8 April 2005

Science

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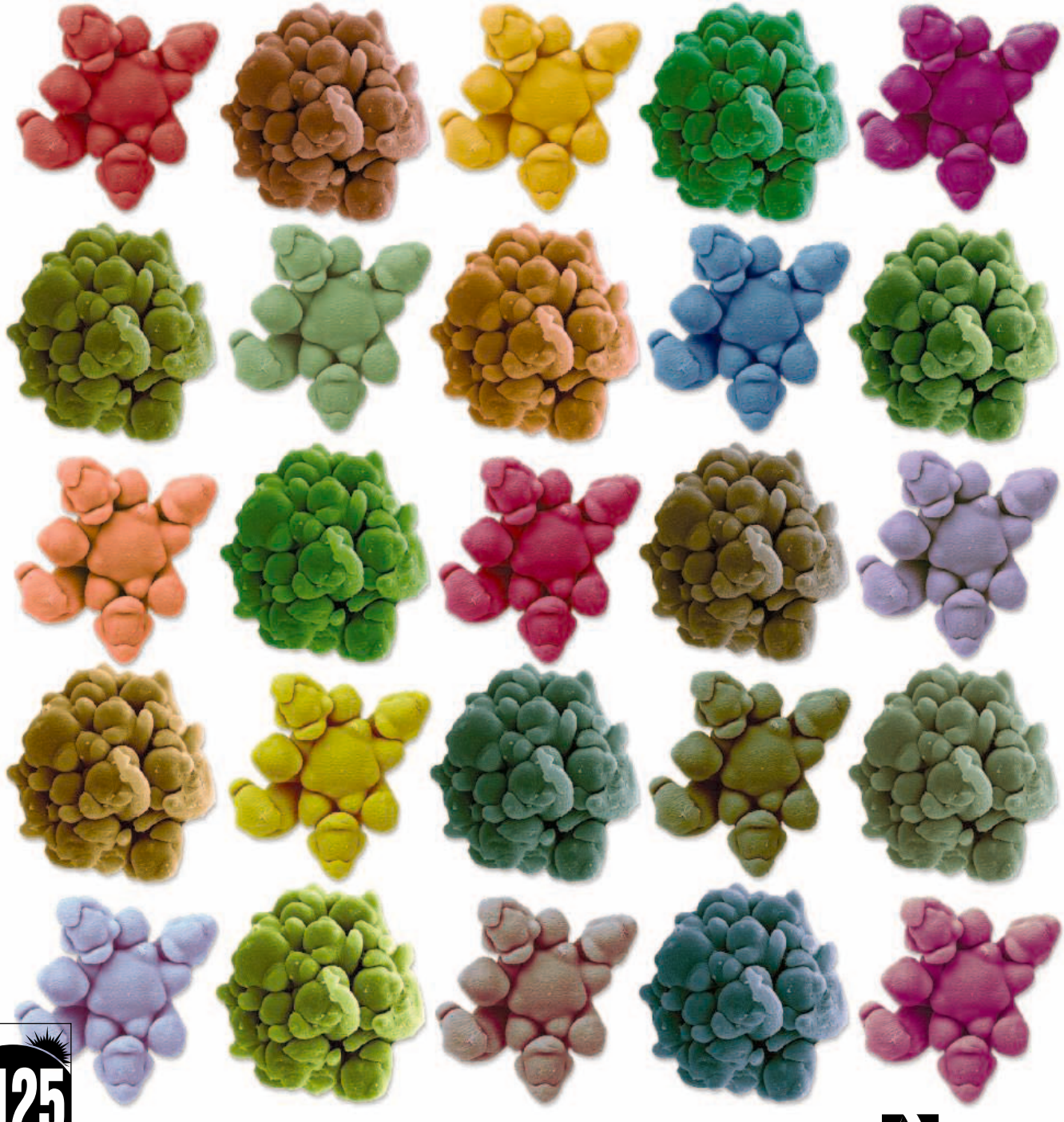
Pages 149–308 $10



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imagination at work

Science

COVER A montage of pseudo-colored *Arabidopsis* inflorescence apices as observed by electron microscopy; wild types and *leafy* mutants alternate, starting with a wild type at the top left. The *LEAFY* gene is critical for acquisition of floral identity in angiosperms and sporophyte development in mosses. Changes in the DNA binding domain are responsible for differences in the biological activity of LEAFY between mosses and flowering plants. See page 260. [Image: A. Maizel and J. Berger]

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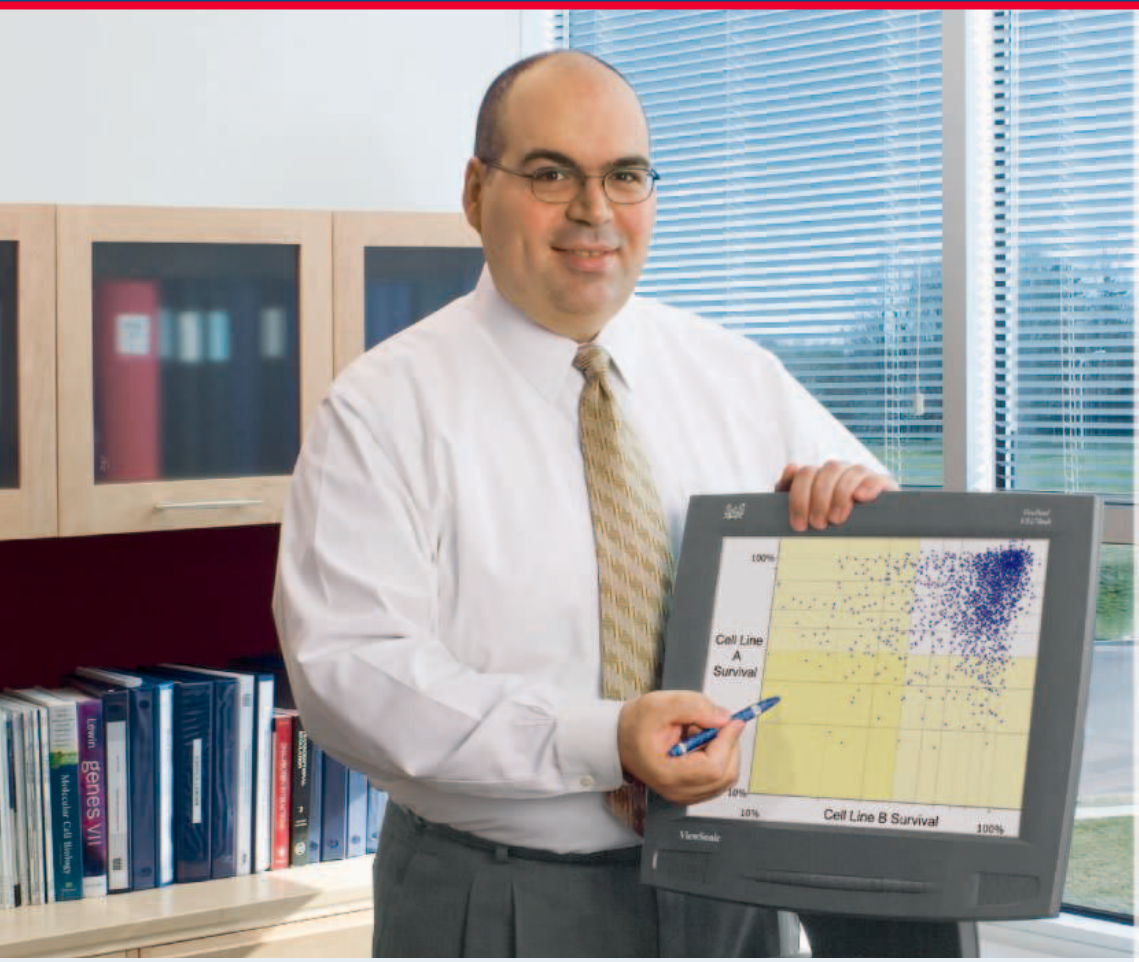
Potent, specific siRNA you can trust!



Spyro Mousses, Ph.D.

Head of Cancer Drug Development, Translational Genomics Research Institute (TGen), Maryland, USA. TGen headquarters are based in Phoenix, AZ.

Dr. Mousses and his colleagues at TGen use siRNA designed by QIAGEN to discover genetic weaknesses in cancer cell lines. In this example, 2000 siRNAs were used in a screen to compare relative survival of two cancer cell lines after gene knockdown.

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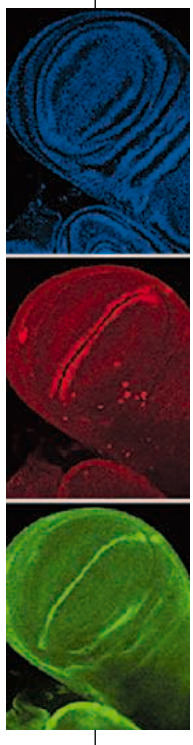

Technical Comment Abstracts

Brevia

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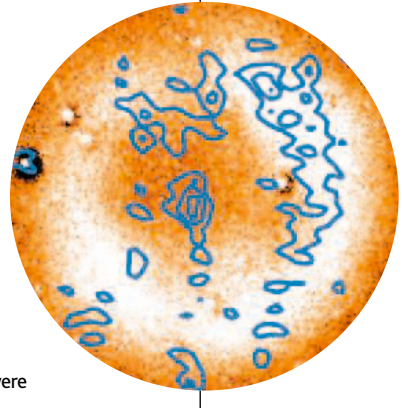

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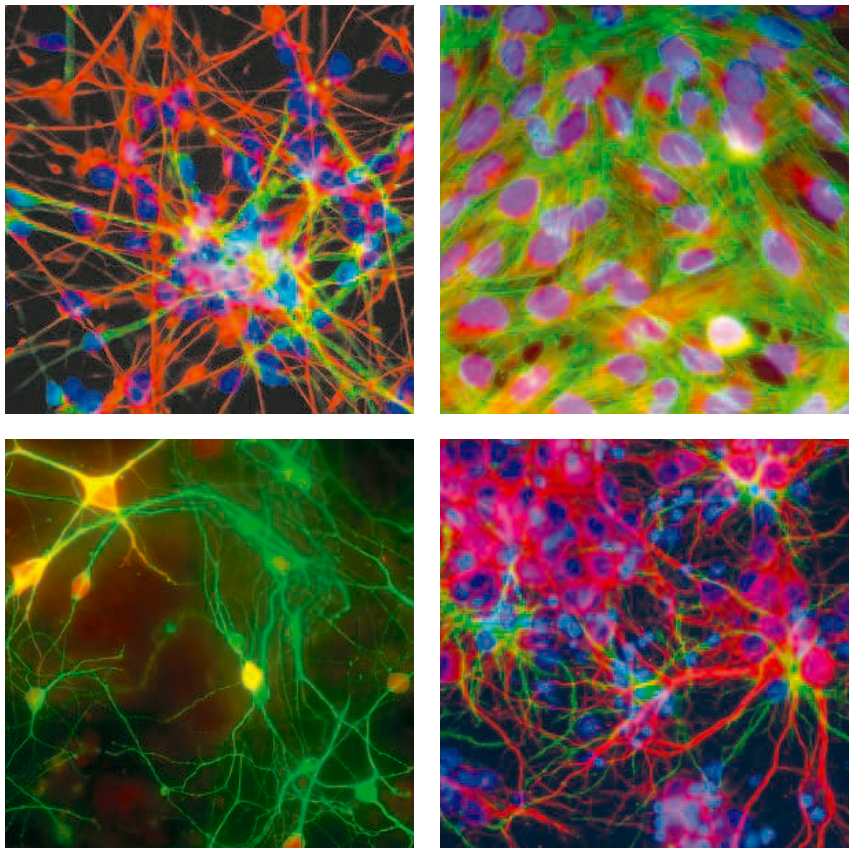

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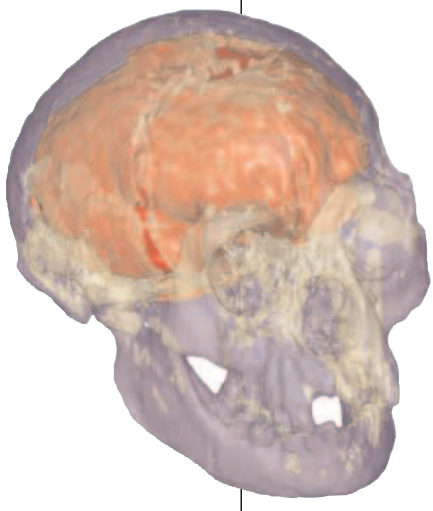

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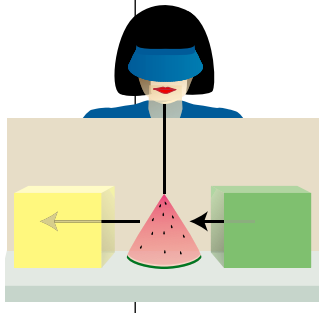

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Molecular scissors help replace defective genes.

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Scientists engineer dairy cows to resist mammary infection.

New Superbug Has Criminal Past
Deadly bacterium now on the rise traced back to epidemic decades ago.

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Careers at the extremes.

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Global: Science Careers in Extreme Environments—Feature Index *E. Pain*
Scientists studying in the Arctic, tropical forests, or on active volcanoes face exciting challenges.

Global/US: Opposite Extremes *J. Kling*
Craig Cary's extremophile research takes him from the hot and wet to the dry and cold.

Global/US: Into the Jungle *R. Arnette*
A wildlife ecologist at the University of Florida in Gainesville talks about his research in the Amazon.

Global/Canada: Life on the Edge—Adventures of an Extremophilic Scientist *A. Fazekas*
A paleogeologist at NASA conducted Mars-analog research at both Poles and the Atacama Desert in Chile.

Global/UK: A Volcanologist's Vista *A. Forde*
Tamsin Mather talks about her fieldwork in exotic locations in Chile, Nicaragua, and Italy.

Career Development Center: Flying Blind *GrantDoctor*
What should you do when your NIH grant program officer won't return your phone calls or e-mails?

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Perspective: Future Mortality—A Bumpy Road to Shangri-La? *S. Tuljapurkar*
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Case Study: Dementia of the Alzheimer's Type and Accelerated Aging in Down Syndrome
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News Focus: Power Cut *M. Leslie*
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News Focus: Giant Steps *R. J. Davenport*
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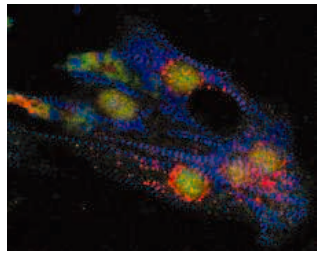
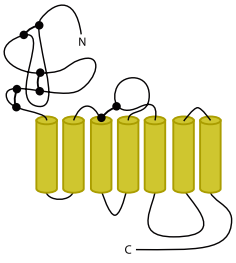


Where titin springs to the muscle's genes.



Glucagon-like GPCR topology.

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Teaching Resource: G Protein–Coupled Receptors *S. C. Sealfon*
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Glossary
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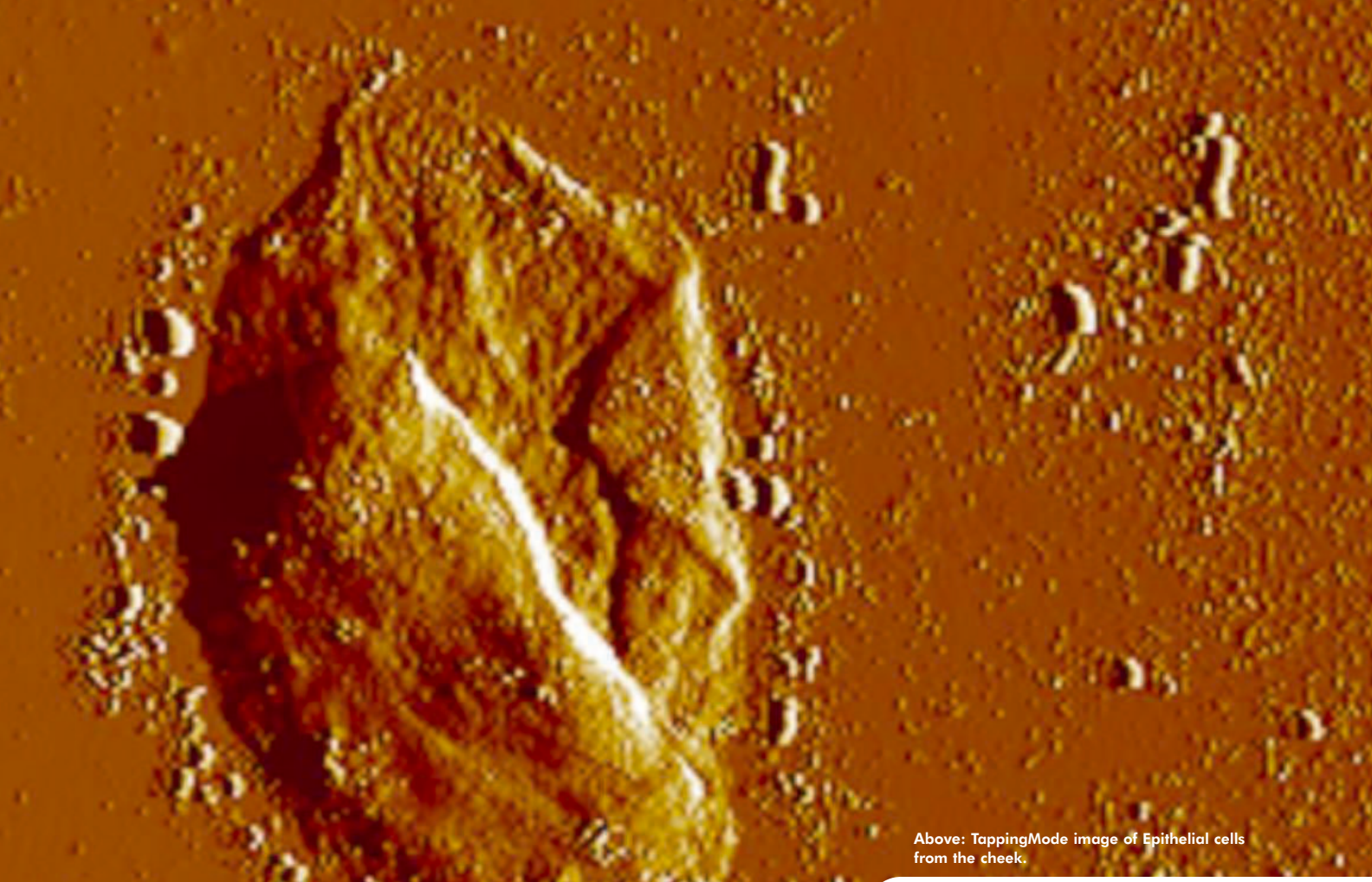

Above: TappingMode image of Epithelial cells from the cheek.

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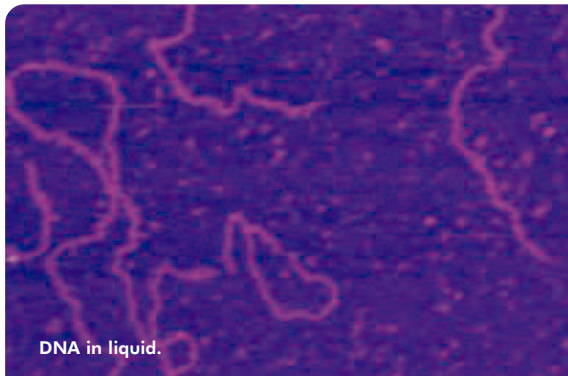

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A Star Is Reborn

As they grow old, stars with approximately the mass of our Sun experience explosive flashes just before their nuclear furnaces shut down. These flashes expel the stars' outer shells and leave behind hot dense remnants called white dwarfs. Most of these remnants simply cool down, but some can experience a late explosion that restarts nuclear burning and expands them into giant stars again. **Hadjuk *et al.*** (p. 231; see the Perspective by **Asplund**) report observations and a stellar model of V4334 Sgr, a "born again" giant that reignited in 1992 and that was discovered by amateur astronomer Sakurai. The subsequent temperature drop of V4334 Sgr is 100 times faster than had been expected. The calculated mass ejection rates suggest that reignition events contribute unexpectedly large amounts of carbon and carbonaceous dust to the interstellar medium.

Through Stick and Thin

Coatings are often used to modify the durability, wettability, or optical properties of a substrate. Typically, one needs to tailor and optimize the chemistry of the material being coated to ensure that it properly adheres to the bulk object. **Ryu *et al.*** (p. 236) show that thin cross-linkable polymer films can be made to adhere to a wide range of substrates, thus presenting a uniform surface onto which further materials can be deposited.

A Snowball's Chance

It has been argued that Earth was covered in ice by glaciers that extended from the poles to the equator as many as four times during the Neoproterozoic (between 750 and 580 million years ago), in what are commonly referred to as "Snowball Earth" periods. Estimates of the length of these episodes (assuming that they actually occurred) range from 100,000 to 30 million years. **Bodiselitsch *et al.*** (p. 239; see the news story by **Kerr**) report independent estimates of the duration of two of these periods, based on the accumulation of extraterrestrial iridium, which indicate that snowball conditions persisted for 3 million to 12 million years.

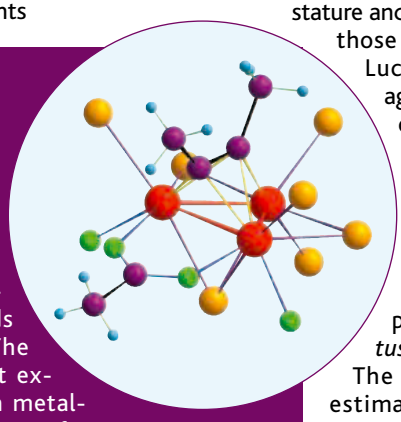
Reheating the Early Solar Nebula

An important heat source in the early solar system was the decay of ^{26}Mg to ^{26}Al. It has also been assumed that early solar system condensates had the same initial $^{26}Al/^{27}Al$ ratio. Because Al is one of the first elements to condense out of the nebular gas into Ca-Al–rich inclusions (CAIs), this decay system has provided perhaps the most important chronometer for dating events between formation of the solar nebula and the first planetesimals. **Young *et al.*** (p. 223) now show that this assumption of a homogenous incorporation of Al in early condensates is not correct, and that the initial ratio was likely much higher. This revision implies that many CAIs reflect a history of much episodic reheating in the solar nebula for several hundred thousand years.

Assessing the Brain of *Homo floresiensis*

Homo floresiensis is the diminutive hominid dated to about 12,000 years ago that was discovered recently in a cave on the island of Flores. Many aspects of this find are puzzling, including its small stature and brain, which is about the size of those of australopithecines such as Lucy's (dated to 3 million years ago). **Falk *et al.*** (p. 242, published online 3 March 2005; see the 4 March news story by **Balter**) have now provided a view of the shape of the brain obtained from a virtual endocast of the skull and compared it with those of other possibly contemporaneous hominids (*Homo erectus* and *Homo sapiens*) and apes. The brain of *H. floresiensis*, now estimated at 417 cubic centimeters, is most like that of a small *H. erectus*, but also has some differences, including a derived frontal lobe and an expanded temporal lobe.

Triple Stitch



During the last decade, alkene metathesis has become a versatile tool in the synthesis of organic compounds and polymers. The reaction is a net exchange, in which metal-catalyzed cleavage of a C=C double bond leads to recoupling of the resulting fragments with a new partner. The analogous reaction of alkynes, or C≡C triple bonds, has been slower to develop and has often required harsher conditions. **Bino *et al.*** (p. 234; see the Perspective by **Bunz**) show that a trimolybdenum cluster, bearing the two separated halves of 2-butyne, reacts in room-temperature aqueous solution to stitch together the alkyne.

Amygdala, Neuropeptides, and Fear Behavior

The neuropeptides vasopressin and oxytocin have opposite effects on fear- and anxiety-related behaviors. At the cellular level, both neuropeptides increase neuronal excitability in different brain regions, including the central amygdala, but the neuronal network underlying these opposite behavioral effects is not yet fully understood. **Huber *et al.*** (p. 245) identified discrete, anatomically separate, populations of oxytocin and vasopressin receptors within the central amygdala. They used oxytocin and vasopressin agonists and antagonists to elicit electrophysiologic neuronal changes and were able to construct a hypothetical neural network in which oxytocin and vasopressin exerted opposing effects on anxiety and fear.

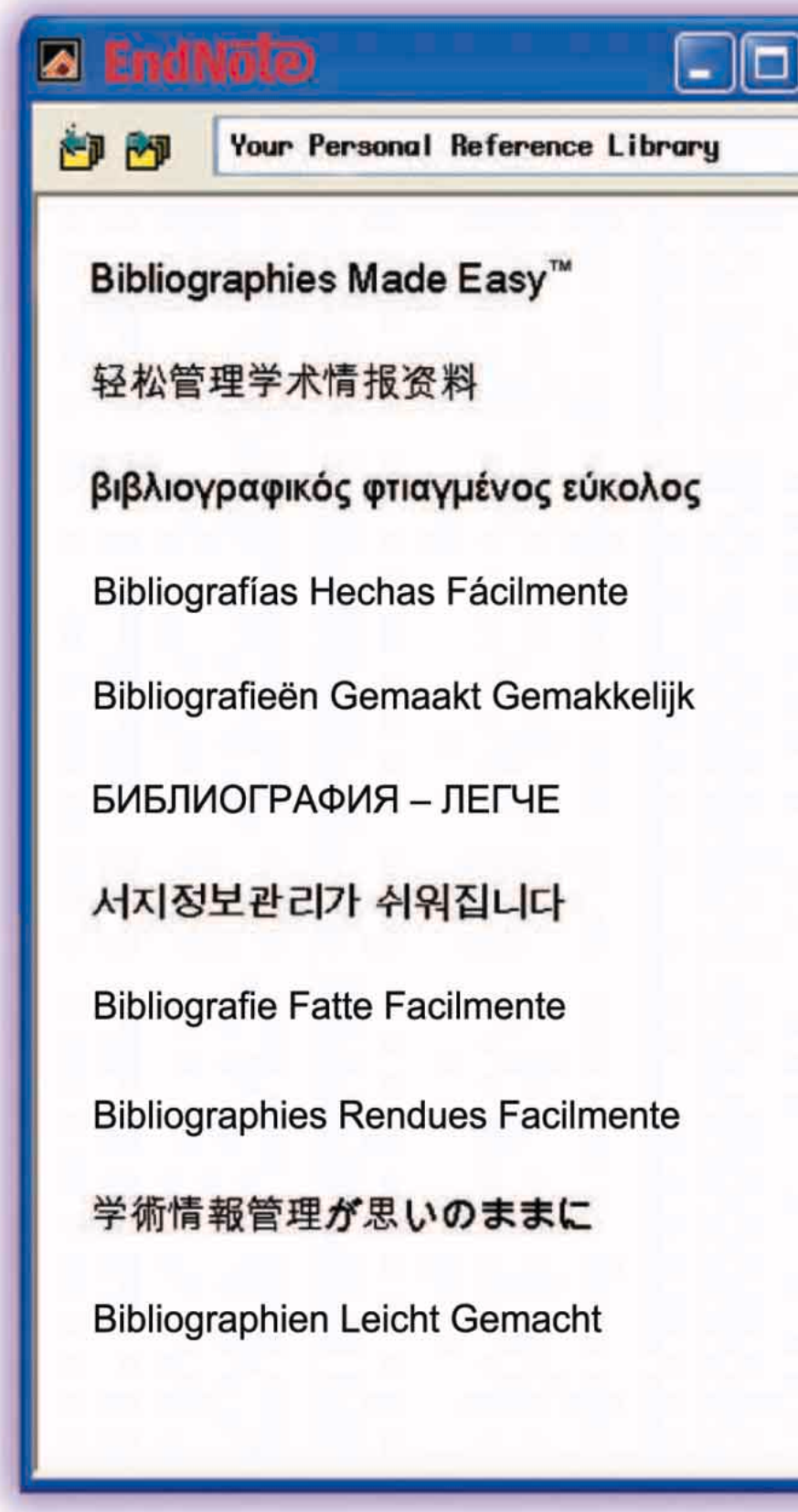
A LEAFY Life-Style Change

The transcription factor LEAFY in flowering plants determines whether key meristems will go on to produce vegetative or floral tissues. In the much more primitive mosses, however, relatives of LEAFY control other aspects of the life cycle. **Maizel *et al.*** (p. 260; see the cover) analyzed the changes in LEAFY sequence and function during this span of evolutionary time. The different functions that LEAFY took on through evolution seem to be attributable to alterations in its DNA binding domain.

CONTINUED ON PAGE 163

CREDITS (TOP TO BOTTOM): BINO *ET AL.*; YOUNG *ET AL.*

Fluent in any language.



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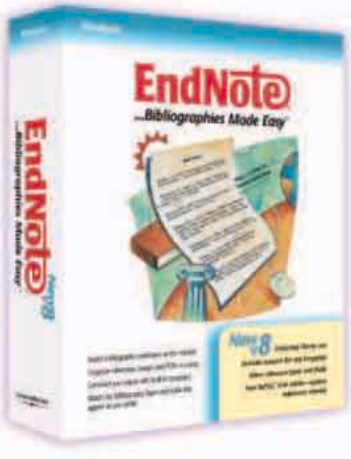

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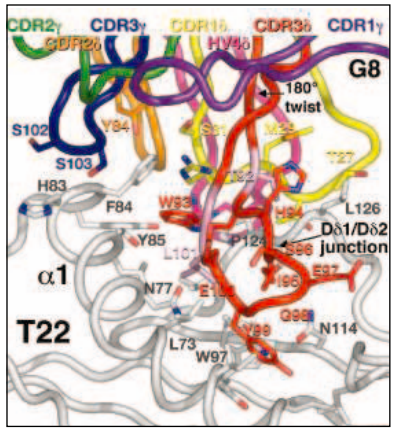
Maintaining Corn Borer Lines

In sympatric speciation, two populations diverge from a common ancestor in the same location. Accurate estimates are lacking of the reproductive isolation between sympatric populations that are genetically differentiated from each other. Using an approach based on isotopic characterization of host plants of two sympatric races of the European corn borer, **Malausa *et al.*** (p. 258) obtained direct field measurements of assortative mating, the key factor of reproductive isolation between sympatric host races. The reproductive isolation between the two races was almost complete, showing that assortative mating can occur in the absence of spatial and temporal isolation.

Earliest Influences

In the immune system, developing thymocytes depend critically on an intimate association with the organized stromal microenvironment of the thymus, which is made up primarily of thymic epithelial cells. **Akiyama *et al.*** (p. 248, published online 11 February 2005) demonstrate the importance of this association for T cell tolerance. A deficiency in the RING domain ubiquitin ligase TRAF6 caused a loss of organized thymic epithelial architecture. The severe impairment of T cell development then leads to autoimmunity.

A New View of an Old Receptor



γδ T cells represent a distinct lineage of T cells that undertake a range of specific immune functions. However, compared with their αβ T cell counterparts, the mode of antigen recognition by these T cells is still relatively poorly understood (see the Perspective by **Garboczi**). By resolving a 3.4 angstrom structure of the complex between a specific mouse γδ T cell receptor (TCR) and its non-classical class I major histocompatibility complex ligand, **Adams *et al.*** (p. 227) have generated a new model of γδ T cell recognition that has features of both innate immune receptor recognition and adaptive recognition through recombination of germline segments. **Shin *et al.*** (p. 252) arrive at similar conclusions from a survey of TCR usage at the single-cell level, which leads them to suggest that γδ T cells focus on a relatively narrow range of antigenic ligands.

Mastering Empathy

Human beings can appreciate that other members of their species think and feel, and that their beliefs and intentions might not correspond with their own. Two decades ago, Wimmer and Perner presented evidence that children do not develop this representational capacity, sometimes referred to as a theory of mind, until ages 3 to 4. **Onishi and Baillargeon** (p. 255; see the Perspective by **Perner and Ruffman**) present evidence that 15-month-old infants may be able to master this task, revealing at the very least an understanding of behavior-action rules.

Eat Your Carrots

The family of carotenoid-synthesizing enzymes includes one that cleaves a 30-carbon precursor (β-carotene) in half to make two molecules of retinal (an essential component of visual pigments) and others that convert β-carotene into the plant hormone abscisic acid and the developmental factor retinoic acid. **Kloer *et al.*** (p. 267) present the structure of one of the enzymes in this family and describe how its active site induces an isomerization in the substrate that helps to position the trans-double bond next to the O_2 molecule used to effect cleavage.

CREDIT: ADAMS *ET AL.*

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	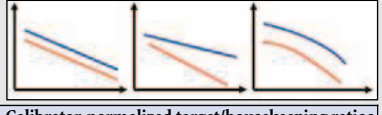		
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Figure 1: Impact of different PCR efficiency adjustments on accuracy of relative quantification. Total RNA was used for quantitative RT-PCR on the LightCycler® System. Sample data were evaluated with the LightCycler Relative Quantification Software, using the efficiency correction functions described above, to generate calibrator-normalized target/housekeeping ratios. The significantly lower Coefficient of Variation (C.V.) demonstrates the greater accuracy made possible by the LightCycler Software's use of efficiency corrections and a non-linear fit function.

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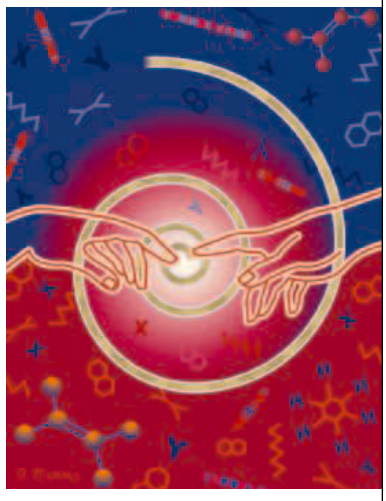
Twilight for the Enlightenment?

For much of their existence over the past two centuries, Europe and the United States have been societies of questioners: nations in which skepticism has been accepted and even welcomed, and where the culture has been characterized by confidence in science and in rational methods of thought. We owe this tradition in part to the birth of the Scottish Enlightenment of the early 18th century, when the practice of executing religious heretics ended, to be gradually replaced by a developing conviction that substituted faith in experiment for reliance on inherited dogma.

That new tradition, prominently represented by the Scottish philosopher David Hume, supplied important roots for the growth of modernity, and it has served U.S. society well, as it has Europe's. The results of serious, careful experimentation and analysis became a standard for the entry of a discovery or theory into the common culture of citizens and the policies of their governments. Thus, scientific determinations of the age of Earth and the theories of gravity, biological evolution, and the conservation of matter and energy became meaningful scientific anchors of our common understanding.



In the United States, that understanding is now undergoing some dissolution, as some school boards eliminate the teaching of evolution or require that religious versions of creation be represented as "scientific" alternatives. "Intelligent design," a recent replacement for straight-up creationism, essentially asserts that a sufficient quantity of complexity and beauty is by itself evidence of divine origin—a retrogression to the pre-Darwinian zoologist William Paley, who saw in the elegant construction of a beetle's antenna the work of a Creator.

In 1998, I helped the National Academies produce a book entitled *Teaching About Evolution and the Nature of Science*. At the press conference announcing its publication, I was asked if I knew that most U.S. citizens did not believe that humans descended from other forms. I said I did, but expressed a hope that things might change. Well, things changed in the wrong direction: Alternatives to the teaching of biological evolution are now being debated in no fewer than 40 states. Worse, evolution is not the only science under such challenge. In several school districts, geology materials are being rewritten because their dates for Earth's age are inconsistent with scripture (too old).

Meanwhile, President Bush's Emergency Plan for AIDS Relief policies recommend "evidence-based" risk-reduction strategies: abstinence for youth, fidelity for married couples, and condoms recommended only for infected or high-risk individuals, such as sex workers. Failure rates for condoms are commonly quoted, apparently to discourage their use by young people for risk prevention. Mysteriously, the policy doesn't seem able to cite a failure rate for abstinence.

Finally, certain kinds of science are now proscribed on what amount to religious grounds. Stem cell research is said by its opponents to pose a "moral dilemma." Yet this well-advertised dilemma does not arise from a confrontation between science and ethical universals. Instead, the objections arise from a particular belief about what constitutes a human life: a belief held by certain religions but not by others. Some researchers, eager to resolve the problem, seek to derive stem cells by techniques that might finesse the controversy. But the claim that the stem cell "dilemma" rests on universal values is a false claim, and for society to accept it to obtain transitory political relief would bring church and state another step closer.

The present wave of evangelical Christianity, uniquely American in its level of participation, would be nothing to worry about were it a matter restricted to individual conviction and to the expressions of groups gathering to worship. It's all right that in the best-selling novels about the "rapture," the true believers ascend and the rest of us perish painfully. But U.S. society is now experiencing a convergence between religious conviction and partisan loyalty, readily detectable in the statistics of the 2004 election. Some of us who worry about the separation of church and state will accept tablets that display the Ten Commandments on state premises, because they fail to cross a threshold of urgency. But when the religious/political convergence leads to managing the nation's research agenda, its foreign assistance programs, or the high-school curriculum, that marks a really important change in our national life. Twilight for the Enlightenment? Not yet. But as its beneficiaries, we should also be its stewards.

Donald Kennedy
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10.1126/science.1112920

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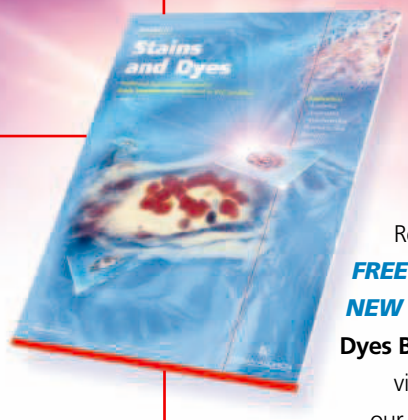

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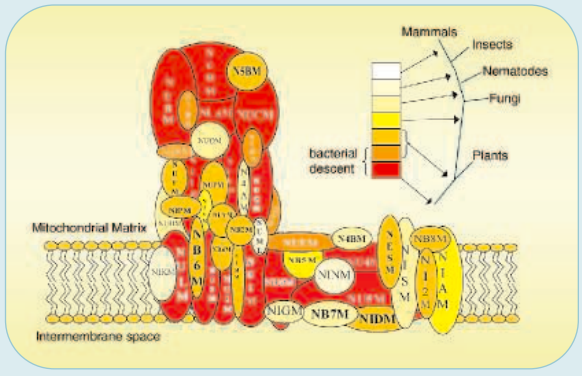
BIOCHEMISTRY

Subunit Arithmetic



Evolutionary origins of complex I subunits.

The largest and least well-described (in structural terms) of the five respiratory enzyme complexes in mammalian mitochondria is the NADH:ubiquinone oxidoreductase, also known as complex I. Only recently has it been determined that the number of distinct subunits is 46, in comparison to 30 and 14 for the corresponding complex I orthologs in plants and bacteria, respectively. Applying a comparative genomic analysis using both nuclear and organellar sequence data, Gabaldón *et al.* show that complex I in the eukaryotic ancestor of the fungi, plants, and metazoa had grown to 35 subunits from the simpler bacterial/archaeal core, having added subunits that came along as the endosymbiont was acquired and stabilized and gaining new recruits from the host. In the subsequent eukaryotic radiation, more subunits have been added to and subtracted from all over the complex, as judged by the three-dimensional maps generated from biochemical and electron microscopic studies. This piecemeal aggrandizement contrasts with the modular assembly of existing multisubunit enzymes into the prokaryotic complex I. — GJC

J. Mol. Biol. 10.1016/j.jmb.2005.02.067 (2005).

MATERIALS SCIENCE

Stabilized by Stress

Thin films are often used as protective coatings against wear and corrosion; nitride films are also used as decorative layers. Typically, these films have a 1:1 metal:nitrogen composition, as in TiN, ZrN, or CrN, all of which have a NaCl-like structure and are highly conductive. Although it is difficult to make films with higher proportions of nitrogen, there have been reports of films with a M_3N_4 composition. Under high pressure inside a diamond anvil cell, the orthorhombic structure of these materials transforms into a metastable cubic structure, and the films become transparent and less conductive.

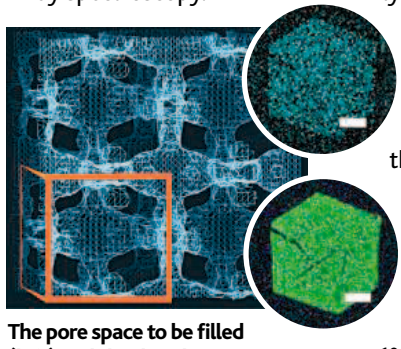
Chhowalla and Unalan have designed an industrially viable, filtered cathodic arc process to create cubic Zr_3N_4 films. Metal vapor is generated by an arc discharge on a pure Zr cathode and reacted with fully ionized atomic nitrogen. The deposition process produces films with inherent compressive stresses and, in combination with the localized high temperatures where the plasma is deposited, creates conditions that stabilize the cubic phase of Zr_3N_4. These films were found to be much harder than either ZrN or orthorhombic Zr_3N_4, and showed excellent wear resistance when used to coat a steel-milling tool. — MSL

Nat. Mater. 10.1038/nmat1338 (2005).

CHEMISTRY

Fixing Nanoscaffolds

Crystals of a virus have been used as templates for making metal-organic nanocomposites. Falkner *et al.* exploited the large void spaces in cowpea mosaic virus crystals (~50% of the crystal) by crosslinking the virus particles with glutaraldehyde. These scaffolds were exposed to tetrachloropallidate(II) ions, which bind to basic amino acids, and then to a buffer containing tetrachloroplatinate(II) ions and sodium hypophosphite. The hypophosphite reduced Pd(II) to the metal, which in turn reduced Pt(II) to the metal. The metallic content of the composite was 10% Pd and 55% Pt by weight, as determined by energy-dispersive x-ray spectroscopy.



The pore space to be filled (left) and the distributions of Pd and Pt (bar, 100 μm).

Transmission electron microscopy revealed that the deposited metal mainly fills in the larger pores but not the smaller spaces connecting them. — PDS

J. Am. Chem. Soc. 10.1021/ja044496m (2005).

IMMUNOLOGY

Sentries at the Portal

The liver is a huge and metabolically active organ, enriched with substantial numbers of non-conventional immune cells that help to protect it from pathogens and potentially harmful immune responses to benign foreign material (such as antigens in food). Particularly striking are the many natural killer T (NKT) cells, which likely serve to regulate hepatic immunity.

To examine the behavior of hepatic NKT cells in situ, Geissmann *et al.* used mice in which one of the alleles coding for the NKT chemokine receptor CXCR6 had been replaced with green fluorescent protein. Intravital confocal microscopy revealed that NKT cells remained confined to the blood vessels within the liver, moving randomly and visiting each hepatocyte every quarter of an hour. This behavior differs from that of conventional activated T cells, which generally pass across the vessel endothelium into the surrounding tissue. Nevertheless, as do T cells on patrol in lymph nodes, hepatic NKT cells stop moving upon encountering antigen, consistent with their surveillance duties. In the absence of CXCR6, the number of hepatic NKT cells was significantly reduced, suggesting that this chemokine receptor mediates a survival signal. — SJS

PLoS Biol. **3**, 10.1371/journal.pbio.0030113 (2005).

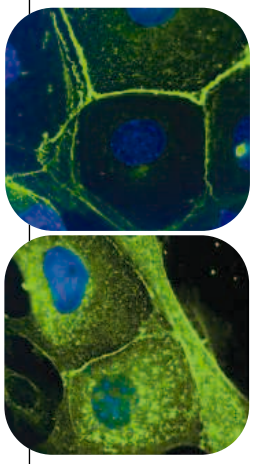
CELL BIOLOGY

Perp Finds Its Purpose

During mammalian development, the single-layered ectoderm surrounding the embryo initiates a stratifica-

CONTINUED ON PAGE 169

CREDITS: (TOP) GABALDÓN *ET AL.*, *J. MOL. BIOL.* 10.1016/J.JMB.2005.02.067 (2005); (BOTTOM) FALKNER *ET AL.*, *J. AM. CHEM. SOC.* 10.1021/JA044496M (2005)



Distribution of the desmosomal protein desmoplakin (green) in the presence (above) and absence (below) of Perp.

tion program that culminates in the formation of the epidermis, an outer barrier that protects the organism from dehydration and environmental insults. The transcription factor p63—a relative of the renowned tumor suppressor p53—plays a critical role in this developmental program, but little is known about its targets or the mechanisms involved.

Ihrie *et al*. show that p63 directly regulates a gene whose product helps epithelial cells stick to each other. This gene, called *Perp*, encodes a membrane protein of the tetraspanin type and is highly expressed in developing skin. Perp localizes to desmosomes, specialized intercellular adhesive complexes that maintain the structural integrity of the skin and are crucial for its strength and resiliency. Newborn mice deficient in Perp display defects in desmosomes, and they die a few days later with severe skin blistering. The authors hypothesize that Perp plays a role in the shuttling, assembly, or stabilization of core desmosomal proteins. — PAK

Cell **120**, 843 (2005).

ENVIRONMENTAL SCIENCE

Water Treatment Plants

Long-term ingestion of low concentrations of arsenic is detrimental to human health, yet in several countries around the world, large populations are constantly exposed to drinking water contaminated with arsenic. In Bangladesh, arsenic concentrations exceed World Health Organization guidelines in 60% of the groundwater. Arsenic can be removed by filtration and via adsorbents, such as natural zeolites, but there still is a need for simple and cost-effective methods using materials that are readily available in developing countries.

Al Rmalli *et al.* show that the dried pulverized roots of the water hyacinth can rapidly remove arsenic from water. The method is effective for both arsenite [As(III)] and arsenate [As(V)] and requires comparatively little material (50 μg of As are adsorbed per g of roots in 24 hours). Water hyacinths grow abundantly in ponds, lakes, and rivers in Bangladesh, India, and other tropical and subtropical countries. The simplicity of the method suggests that these plants may be useful in the treatment of drinking water, particularly in rural areas. — JFU

J. Environ. Monit. **7**, 279 (2005).

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

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Sensing a Need for Oxygen

The sterol regulatory element–binding proteins (SREBPs) are a family of endoplasmic reticulum (ER) membrane-bound transcription factors that, in mammals stimulate the transcription of genes involved in cholesterol and fatty acid synthesis, and are regulated by feedback inhibition. SREBP is activated through proteolytic cleavage in the Golgi; high concentrations of sterols promote formation of a complex between SREBP and the SREBP cleavage-activating protein (SCAP) and the ER protein Insig, which traps SREBP-SCAP in the ER. Hughes *et al.* identified a gene (*sre1*$^+$) in fission yeast with sequence similarity to that encoding human SREBP-1a and a similar membrane topology, and also the yeast homologs of SCAP (*scp1*$^+$) and of Insig-1 (*ins1*$^+$). Microarray analysis of sterol-depleted wild-type yeast and yeast lacking *scp1* indicated that Sre1 promoted the transcription of genes involved in sterol biosynthesis and also that of genes required for the shift from aerobic to anaerobic growth. Yeast lacking *sre1* or *scp1* were unable to grow in the absence of oxygen, whereas low oxygen stimulated the expression of Sre1 targets in wild-type cells. Shifting yeast to low oxygen reduced ergosterol synthesis; after several hours, wild-type cells were able to adapt and increase ergosterol synthesis, whereas cells lacking *sre1* could not. Thus, the authors propose that Sre1 monitors oxygen availability through oxygen-dependent sterol synthesis. — EMA

Cell **120**, 831 (2005).

CREDITS: IHRIE *ET AL.*, *CELL* **120**, 843 (2005)

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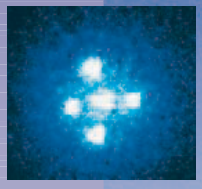

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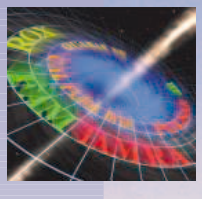

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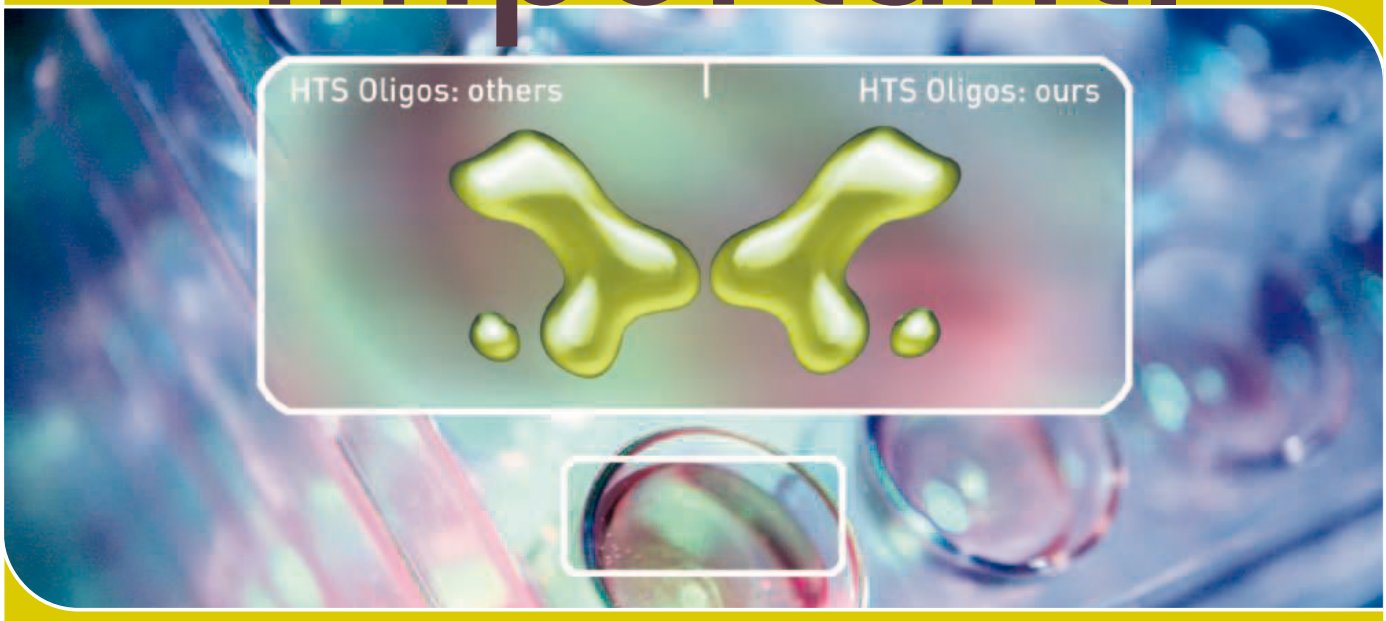

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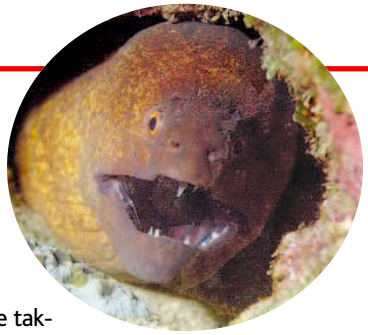
NetWatch

edited by Mitch Leslie



DATABASE

Coral Case Files

The world's reefs are taking a beating from silt buildup, explosives used for fishing, the coral-munching crown-of-thorns starfish, and other threats. Consult this database to find out the condition of individual reefs—some 70% of which are ruined or in jeopardy. Reef Check, a nonprofit based in Pacific Palisades, California, collates reports from volunteers who collect standard data on coral health (*Science*, 6 September 2002, p. 1622). Click on the barometer at the bottom of the Reef Check home page to search more than 3400 surveys from 1800-plus locales around the world. The records provide information such as ratings of natural and human-caused damage and counts for fishes, invertebrates, and other residents. Tools let you compare reefs and contrast measures of the same site from different times. Above, a moray eel gapes from the safety of a crevice.

www.reefcheck.org

EDUCATION

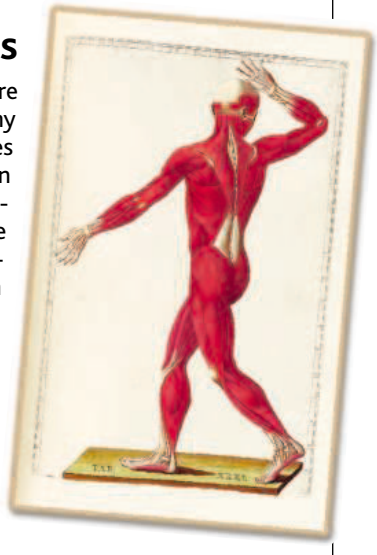
Oyez, Oyez

Whether you're curious about why music sounds better in some rooms than in others or you want to learn how to derive the equations for the Doppler shift, tune in to this site from retired engineer Art Ludwig of Santa Barbara, California. Although short on graphics, the site is well stocked with informative text explaining the physics of sound for beginners and experts. The mathematically adept can learn how to work out the sound equations from a model of molecular motion. Or delve into image analysis, which is useful for evaluating the acoustics of a room. Math-free sections explore topics such as the fundamentals of sound and how we hear music.

www.thesoundpage.com

EXHIBITS

Anatomy Through the Ages



If not for its jaunty pose, this muscular figure (right) would look at home in a modern anatomy textbook. But the drawing comes from a series of plates commissioned by the Italian physician Bartolomeo Eustachi in the mid-1500s and published some 150 years later. Readers can see these and more lush medical illustrations at Historical Anatomies from the U.S. National Library of Medicine. The continuing exhibit showcases selected diagrams from 28 anatomical atlases, spanning a 14th century Persian treatise to a 19th century German book on frozen cross sections. Brief backgrounders highlight the innovations in each work and describe the authors. For example, Eustachi (circa 1500–1574) was a traditionalist and opposed the upstarts who were challenging the ancient Roman anatomist Galen, then considered the ultimate authority on the body's structure.

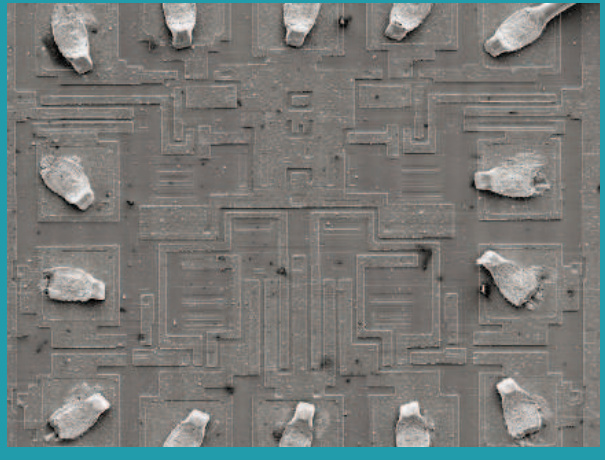
www.nlm.nih.gov/exhibition/historicalanatomies/home.html

IMAGES

Scope This Out

Many beginning students never get closer to an electron microscope than the photos in their textbooks. But anyone can get a sense of what the instrument can do by downloading this simulator from NASA's Kennedy Space Center. The free Java program allows users to pan, zoom, and use the built-in ruler to measure a beetle's leg, crystals from a human kidney stone, and other objects. Below, an integrated circuit. The virtual lab plans to add more images and instruments, such as a light microscope, says project leader Berta Alfonso.

learn.arc.nasa.gov/vlab/index.html



RESOURCES

Bug Basics

Looking for a clearer description of the structure of the cell skeleton? Need a simple procedure students can use to isolate *Streptococcus* bacteria? Click over to the Grapes of Staph, a combination Web text and lab manual from microbiologist Gary Kaiser of the Community College of Baltimore County in Maryland. Although tailored for Kaiser's classes, the site offers plenty of material that other teachers can adopt. The tutorial includes more than 50 sections on basic microbiology, covering everything from bacterial anatomy to viral life cycles to the human body's defenses against invading microbes. Readers will also find illustrations and animations, a glossary, and self-quizzes. The 22 lab exercises teach students techniques for culturing and isolating bacteria, testing for pathogens, and more.

www.cat.cc.md.us/~gkaiser/goshp.html

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

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This Week

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Our oldest ancestor?

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Evidence for snowball Earth

U.S. PATENT LAW

Case Probes What's Fair Game In the Search for New Drugs

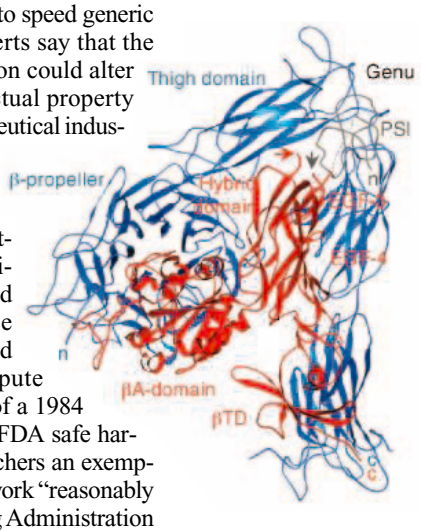
How much freedom does a drug company have to use someone else's patented research tools in the course of developing new therapies? This month, the U.S. Supreme Court will hear oral arguments in a patent case between a German pharmaceutical giant and a small U.S. biomedical company involving a 20-year-old federal law originally passed to speed generic drugs to market. Legal experts say that the court's answer to that question could alter the ground rules for intellectual property claims not just in the pharmaceutical industry but also across many areas of basic research.

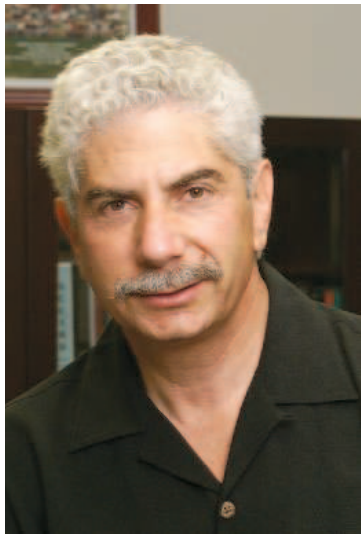
On 20 April, the high court will hear arguments pitting Merck KGaA (not affiliated with the U.S.-based Merck & Co.) against the Plainsboro, New Jersey–based Integra. The 9-year-old dispute rests upon an interpretation of a 1984 federal law—known as the "FDA safe harbor"—that gives drug researchers an exemption from patent liability for work "reasonably related" to the Food and Drug Administration (FDA) approval process. The outcome of the case will hinge on the court's definition of "reasonably related" research. Merck's position is supported in briefs filed by the U.S. government, big pharma, and the seniors' lobbying group AARP. Arrayed on the other side are small and large biotech companies that primarily sell patented tools to scientists.

Congress created the safe-harbor exemption in 1984 so that companies making generic drugs could work with patented materials in preparing FDA applications without having to wait until the patent expired. Federal courts have since steadily expanded the kinds of research covered by the exemption to include research on novel drugs and medical devices.

The trigger for the legal battle was a 1994 discovery by David Cheresh, a cell biologist at the Scripps Research Institute in La Jolla, California, that molecules that bind to a surface protein called αvβ3 inhibit blood vessel growth, or angiogenesis (*Science*, 22 April 1994, p. 569). Merck soon cut a deal with Cheresh and Scripps to begin to identify drug candidates in animal models and, as the agreement stated, "satisfy … (FDA) regulatory requirements."

A small firm called Telios, though, held patent rights on the use of peptides containing a particular sequence, known as RGD, that binds to αvβ3. Cheresh's work involved RGD peptides. Telios sued in U.S. District Court, soon after which Integra bought the intellectual property from Telios. In 2000, the jury found that at least one and as many as 180 Merck experiments between 1994 and 1998—most involving testing how effectively several compounds starved tumors on chicken embryos—had infringed Integra's patents and awarded Integra $15 million. An appellate court upheld the ruling in 2003, calling Cheresh's work "preclinical" and not tied to FDA approval of any drug. By narrowing the exemption's scope, says patent attorney Denise DeFranco of Foley Hoag LLP in Boston, the ruling "kind of shook up some established expectations."





Trigger. David Cheresh's discovery that blocking αvβ3 (*top, left*) inhibits angiogenesis led to a search for new drugs—and a patent dispute.

Not according to Integra's attorneys, who say that the appellate court was simply affirming the rights granted to any patent holder. "The biotech industry is all about new tools," says Mauricio Flores, an attorney for Integra at McDermott Will & Emery LLP in Washington, D.C. In a supporting brief, biotech companies Affymetrix and Invitrogen say that a broad interpretation of the safe harbor provision would undermine their ability to earn licensing revenue for innovations that lay the groundwork for new medicines.

The appeals court ruled that the Scripps-Merck experiments amounted to "new drug development activities." Integra lawyers point out that chicken embryos—unlike mice, for example—are not considered predictive enough of human health for FDA requirements. Merck is trying to use the safe harbor "as a cover for [patent] infringing work," Integra argues in its brief. But Merck says the embryo work addressed FDA requirements regarding efficacy and other metrics. Merck maintains that the studies came after Cheresh had "a viable drug candidate" in hand and that the safe harbor "embraces any information that a drug innovator could reasonably expect to submit to the FDA in connection with *any* application."

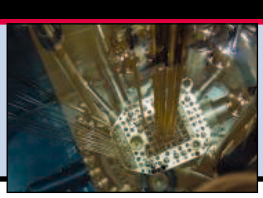
Several biotech attorneys believe that the appeals court erred in ruling that Cheresh's "preclinical" work was not part of the FDA approval process. "Limitation of the safe harbor to 'clinical' experiments would ignore the extensive preclinical data required by the FDA," argues the American Intellectual Property Law Association in its brief.

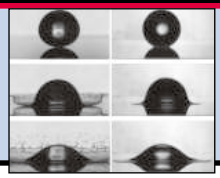
Some patent attorneys are worried that the high court, regardless of its ruling on the safe harbor, might narrow a related practice that provides academics and others pursuing basic research with an exemption for "experimental use." Courts have narrowed its applicability over the last 2 decades, most recently in a case that pitted Duke University against a former professor, laser scientist John Madey (*Science*, 3 January 2003, p. 26).

An appellate judge mentioned the research exemption in her dissent, raising the possibility that the high court could inadvertently confirm a restrictive new standard. "That's the worst fear," says Josh Sarnoff, a law professor at American University in Washington, D.C., and an attorney for the Consumer Project on Technology, which has filed a brief in support of Merck's position.

–ELI KINTISCH

CREDITS (LEFT TO RIGHT): J. P. XIONG *ET AL.*, *SCIENCE* **293**, 1745 (2001); THE SCRIPPS RESEARCH INSTITUTE





INFECTIOUS DISEASES

North Korea Collaborates to Fight Bird Flu

World health officials are encouraged by two developments in North Korea. First was confirmation earlier this week that an outbreak of avian influenza in that isolated country was not due to the dreaded H5N1 subtype. Second, and perhaps equally important for long-term efforts to monitor emerging diseases, was North Korea's willingness to cooperate with global animal and human health surveillance efforts. The country notified the United Nations Food and Agricultural Organization (FAO) of the outbreak and allowed FAO experts to confirm the virus as an H7 subtype. Korean officials also informed the World Health Organization (WHO). Such exchanges, unthinkable a decade ago, suggest that the secretive nation is increasingly willing to participate in global health protection efforts.

"I think they know the seriousness of avian influenza, so I'm rather positive in hoping for a continuation of [cooperation] in the future," says Hans Wagner, senior animal health officer for FAO's regional office in Bangkok.

Such cooperation is vitally important in watching for any evolution in the H5N1 virus. Since December 2003, the virus has devastated poultry flocks throughout Asia and has claimed at least 49 human lives. Almost all human infections have been traced to contacts with infected poultry. But health officials worry that if the H5N1 virus acquires the ability to spread easily among humans, it could set off a deadly pandemic.

Despite a long history of self-imposed isolation, North Korea has increasingly been cooperating with international agencies over the past decade. The World Food Program and WHO have both expanded their work in the country since they opened offices there in 1995 and 2001, respectively. And even before North Korea had suffered any major outbreaks of avian flu, last fall it joined an FAO network of east Asian countries set up to cooperate in fighting H5N1, says Wagner. North Korean veterinary officials have attended regional FAO symposia on avian influenza, and FAO is planning a May workshop on surveillance for North Korean animal health officials.

North Korea officially informed the FAO regional network of the avian influenza outbreak on 27 March, 2 weeks after rumors appeared in the South Korean press. Wagner, who arrived on 29 March, says North Korean officials allowed an FAO expert to verify tests suggesting the virus was an H7 subtype. Wagner's hosts also took him to the index farm and described their culling methods. "We can work with and collaborate with DPRK authorities," Wagner says.

Diego Buriot, a WHO special adviser on communicable diseases, calls North Korea's decision to request technical assistance from FAO "a very positive step," adding that "WHO is also ready to provide expertise if requested by the government."

Wagner is counting on further cooperation. Although reassured that the virus is not H5N1, he notes that H7 subtypes remain a serious threat to poultry—and thus to food availability—in the impoverished country. An H7N7 avian flu outbreak in the Netherlands in 2003 claimed one human life and sickened dozens. Wagner says the specific N subtype of the North Korea virus has yet to be determined, but this is the first recorded instance of an H7 virus in Asia. FAO experts will be working with their North Korean counterparts to try to trace where the virus came from and how to prevent further spread. The exercise may turn out to be a dry run in case H5N1 does turn up in North Korea. –DENNIS NORMILE

CONFLICT OF INTEREST

Scientists, Societies Blast NIH Ethics Rules

New ethics rules at the National Institutes of Health in Bethesda, Maryland, unfairly punish all employees for the sins of a few and will isolate NIH researchers from the scientific community. That's the gist of roughly 1000 comments by NIH employees and many scientific societies submitted by a 4 April deadline to the Department of Health and Human Services (HHS) in Washington, D.C. Even before the comments were in, a handful of top NIH researchers had announced that the rules had prompted them to leave NIH. The latest is National Institute on Deafness and Other Communication Disorders Director James Battey, who says a ban on owning biomedical stocks put him in an impossible position (see p. 197).

NIH Director Elias Zerhouni unveiled the strict new HHS ethics regulations in February after questions arose about a handful of NIH researchers who were consulting for companies. Many comments say reforms were needed, but the rules go too far. They are "not carefully aligned with risk" and "will have a significant negative impact on the progress of biomedical research as a whole," writes the Federation of American Societies for Experimental Biology (FASEB) in Bethesda, Maryland. Thirty-nine of NIH's 43 National Academy of Sciences members signed a letter calling the rules "unfair," "totally unjustified," and "a serious threat to the intramural research program."

The stock rule has drawn the most fire. It bans about 6000 senior employees and their families from owning any stock in drug, biotech, or medical-device companies. Others can hold no more than $15,000 in a single medical company. Hundreds of comments, including some from researchers at universities, argue that the order to divest will cause financial hardship for many employees, as well as dissuade outside researchers from coming to NIH. The Association of American Medical Colleges (AAMC) in Washington, D.C., urges NIH to tailor the prohibition to officials with decision-making power, as proposed by NIH's Assembly of Scientists (homepage.mac.com/assemblyofscientists).

A smaller number of comments question the rule's ban on consulting for industry. One NIH scientist noted with chagrin that he had to turn down a company's request to help figure out the mechanism of a new psoriasis drug, a project unrelated to his work.

Scientific societies worry about restrictions on NIH scientists' professional activities and call for the rules to be revised quickly or withdrawn. FASEB and AAMC, for instance, argue that rules banning service on boards shouldn't apply to society boards. They also say NIH scientists should be allowed to give single lectures, which are common at medical schools. These and ▶

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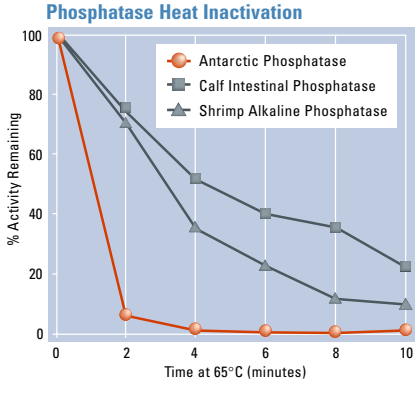

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other organizations urge NIH, which now strictly limits cash awards of more than $200 to "bona fide" awards, to complete that list; the lack of guidance has already caused problems for some societies. A coalition of advocacy nonprofits called the Cancer Leadership Council in Washington, D.C., which relies on voluntary service from NIH employees, argues that it should be exempt, too.

A recent e-mail from NIH's Assembly of Scientists underscores the breadth of the rules, noting that NIH approval is now required for "any outside employment, whether or not for compensation, or any self-employed business activity," even singing in a choral group or selling artwork: "It suggests the NIH owns our lives away from work."

HHS has already made a few slight changes to the rules, exempting temporary researchers from the stock limits and extending until October the deadline for divesting. The agency has said it expects to make any further revisions by next February.

–JOCELYN KAISER

LIVERMORE NATIONAL LAB

Settlement in Bias Case Could Unravel

Lawrence Livermore National Laboratory has agreed to pay a total of $1.2 million to settle a suit alleging discrimination against Asian-American scientists and engineers at the lab. But several plaintiffs say the settlement is too small and discriminates against women; it could be scrapped if enough scientists reject the terms.

The lawsuit, filed in 2001, alleges unfair treatment and inequalities in salary and promotion based on race. The class-action suit was filed by nine individuals representing 460 current and retired employees at the California weapons lab, which is run by the University of California (UC) for the Department of Energy (DOE). Under the settlement agreement, which was approved on 22 March by the Alameda County Superior Court, the employees would split $765,000 and UC would also pay up to $350,000 of their legal costs. Each of the nine persons named in the original suit would get an additional $15,000. The settlement does not admit that discrimination occurred, and Livermore officials declined comment.

Wrong choice. Kalina Wong says the Livermore settlement makes people pick "whether you're a woman or you're an Asian."

CREDIT: K. WONG

Five of the nine named plaintiffs have already rejected the terms as too stingy. Michael Sorgen, the attorney for those five, says the average compensation of $1700 per employee is "paltry compared to the years of depressed salaries and blocked promotions" for many workers. One particularly egregious element, Sorgen says, is that most female employees, who make up roughly a quarter of the class, wouldn't be able to collect anything. That's because the settlement stipulates that any sums awarded to individuals from an earlier sex-discrimination suit, which UC settled in December 2003, would be deducted from the amount they are to receive in this case. In the sex-discrimination suit, UC agreed to pay $10 million to 3200 women who had worked at the lab during a 6-year period and give a 1% raise to 2500 current female employees (*Science*, 5 December 2003, p. 1641).

One plaintiff, Kalina Wong, says the settlement represents continuing discrimination against women. "They're saying you can face discrimination as an Asian, you can face discrimination as a woman, but if you're both, you must pick whether you're a woman or you're an Asian," says Wong, a computer scientist who retired from the lab in 2002. She's the only woman among the nine plaintiffs and the only retiree opposed to the settlement.

The four backing the settlement—all retirees—say they are exhausted after 3 years of litigation. "The lab would just have continued dragging its feet," says Richard Yamauchi, a programmer. He also concluded that the lawsuit would not alter what he sees as a discriminatory environment. "Asian employees will continue to be discriminated against because the people who discriminated against us are still there, and they are passing on their behaviors to future managers," he says. "There is nothing to break the cycle."

Apart from the financial issues, the settlement calls for "a pay, promotion, and rank equity study" of Asian scientists and engineers at the lab every year for the next 3 years.

The affected workers must decide by 31 May whether to participate in the settlement, which goes back to the judge on 21 June. If 10% of them opt out, the university has the right to pull out, too. Sorgen says his clients plan to press ahead regardless of the outcome.

–YUDHIJIT BHATTACHARJEE

ScienceScope

Ban Urged on Smallpox Studies

Two advocacy groups have launched a campaign to halt new studies on variola, the virus that causes smallpox. The Sunshine Project in Austin, Texas, and the Third World Network, headquartered in Penang, Malaysia, are urging the World Health Assembly (WHA), the supreme body of the World Health Organization (WHO), to ignore an expert panel and set a firm deadline for the destruction of the two remaining stocks during its annual meeting in Geneva in May.

In November, WHO's Advisory Committee on Variola Research recommended that work on variola continue and that researchers be allowed to insert a marker gene into the virus—to facilitate drug discovery—and to exchange genes of the variola genome and splice them into other poxviruses to study their function. On a new Web site in six languages (www.smallpoxbiosafety.org), the two groups claim that the work could lead to accidental releases of the agent or to the creation of even more dangerous viruses.

Although experts have long fought over whether to study or destroy variola (*Science*, 15 March 2002, p. 2005), it's rare for outsiders to enter the debate, notes smallpox expert Jonathan Tucker of the Monterey Institute of International Studies in Washington, D.C. The campaign's success may hinge on its ability to attract press attention before the WHA meeting, he adds.

–MARTIN ENSERINK

Los Alamos Bidding Heats Up

Lockheed Martin announced last week that it would bid for management of Los Alamos National Laboratory in New Mexico, adding a solid contender to the fight. The news came after the Department of Energy revised the proposed language of the multibillion-dollar contract in February to require the new contractor to create a new corporate entity and separate pension fund. Lockheed, which manages Sandia National Laboratories in Albuquerque, New Mexico, had previously dropped out, citing costs.

Adding intrigue, last month, current lab manager University of California (UC) announced a possible bid with three New Mexico universities. Although considered the 400-pound gorilla in the contest, UC hasn't made a final decision. The University of Texas is also interested. No final bid date has been set, but UC's existing contract expires 30 September.

–ELI KINTISCH

POSTDOCS

Care and Feeding Pays Off, Survey Finds

Careful tending of a young plant, every gardener knows, is more likely to yield a bountiful harvest. A new survey of 7600 postdocs shows that axiom also applies to the research lab.

The survey, conducted by the scientific society Sigma Xi and released this week (sigmaxi.org), suggests that a well-structured environment for postdocs pays off in greater productivity, more so than do salary and benefits. That finding, says its author, backs up the point that postdoctoral associations and their advocates have been making for years: Treat us with respect, rather than just as a pair of hands, and we'll deliver.

"What really counts is not the money but the [postdoc] experience," says Geoff Davis, who led the study. "[Principal investigators] need to realize that they are obligated to provide training to their postdocs, and universities and funding agencies need to ensure that PIs are fulfilling that obligation."

With support from the Alfred P. Sloan Foundation in New York City, Davis and his colleagues e-mailed a questionnaire to roughly 40%—some 22,000—of the postdocs working at 46 institutions. The list included 18 of the top 20 U.S. academic employers and the largest government employer, the National Institutes of Health in Bethesda, Maryland. Administrative oversight included elements such as an office to manage postdoctoral affairs and formal review of postdocs' performance. Davis also asked about formal and informal avenues for honing postdocs' communication, management, and other professional skills.

Management advantage. Structured oversight has a greater impact on postdoc performance than does salary.

The researchers then correlated these measures with the number of peer-reviewed publications and similar indices of success. The results showed that there was a stronger correlation between administrative oversight and productivity than there was between salary and productivity. "Having a comprehensive career plan, formal reviews, and good training produces an improvement in the postdoc's satisfaction level that is equivalent to a $20,000 raise," says Davis. Postdocs in well-structured positions also seem to report fewer conflicts with their advisers.

The findings reaffirm what postdocs have been saying for a decade, says Alyson Reed, executive director of the National Postdoctoral Association in Washington, D.C., one of the sponsors of the study. "Postdocs make a financial sacrifice in the hope of advancing their career prospects," she says. "They have a greater chance of achieving that goal if they sit down with the PI to develop a formal plan and then use that plan to review progress."

A handful of institutions already have policies to foster that kind of experience, and others are recognizing their value. "It's to everyone's advantage: the institution, the PI, and the postdoc," says Joan Lakoski, a senior administrator at the University of Pittsburgh Health Sciences Schools in Pennsylvania, which this fall will require its PIs to prepare an "Individual Development Plan" for their postdocs. PIs will also have to conduct an annual performance review. "The process could easily be viewed as another tiresome piece of paperwork mandated by the university," says Lakoski. "But it's really an opportunity to improve scientific productivity."

—YUDHIJIT BHATTACHARJEE

POSTDOCS

IRS Takes Bite out of NIH Fellows' Paychecks

Almost 5000 postdoctoral scholars supported by National Institutes of Health (NIH) fellowships took a 7.65% pay cut this week, thanks to a new U.S. tax regulation. The change, which also requires their institutions to pay more to the government, exacerbates a long-running disagreement between NIH and the Internal Revenue Service (IRS) over the employment status of this slice of the U.S. postdoc population.

The IRS regulation, which went into effect 1 April, puts the squeeze on postdocs funded by the Ruth L. Kirschstein National Research Service Awards (NRSA) and some other fellowships by redefining who qualifies for a student exemption that shields the income earned from certain campus jobs. The rule requires employers, mostly universities, to begin withholding FICA (Social Security) and Medicare taxes from their paychecks.

Historically, NIH has argued that its NRSA postdocs are trainees rather than employees. Under that classification, universities do not have to offer postdocs employee group health insurance and other typical work benefits. A NIH spokesperson estimated that there were 4700 postdocs on NIH training grants and similar fellowships in fiscal year 2004. The new rule does not affect the estimated 50,000 postdocs paid out of R01s and other research grants to principal investigators, who have long been classified as employees, with FICA and Medicare taxes deducted from their pay.

In statements on its Web site, NIH makes it clear that it disagrees with the new rule. It maintains that Kirschstein NRSA postdocs are not employees and that the new IRS regulation should not apply to them. The statement goes on to add that "it is, therefore, inappropriate and unallowable for institutions to charge costs associated with employment (such as FICA, workman's compensation, or unemployment insurance) to the fellowship award." It also makes clear who is actually calling the shots. "NIH takes no position on the status of a particular taxpayer, nor does it have the authority to dispense tax advice," the NIH Web site explains. "The interpretation and implementation of the tax laws are the domain of the IRS."

Most universities appear to have gotten the message. "We immediately talked to our internal counsel and then our outside counsel, [who say] all postdocs, medical residents, and medical interns have to pay FICA," says Joel Oppenheim, senior associate dean for biomedical sciences at New York University School of Medicine. Paying the employer's share of FICA and Medicare taxes for the medical school's 300-plus postdocs, he notes, about $3300 per individual, also affects the university's bottom line. "That's over a million bucks a year for us," says Oppenheim.

—BERYL LIEFF BENDERLY

Beryl Lieff Benderly covers postdoctoral issues for *Science*'s Next Wave (www.nextwave.org).

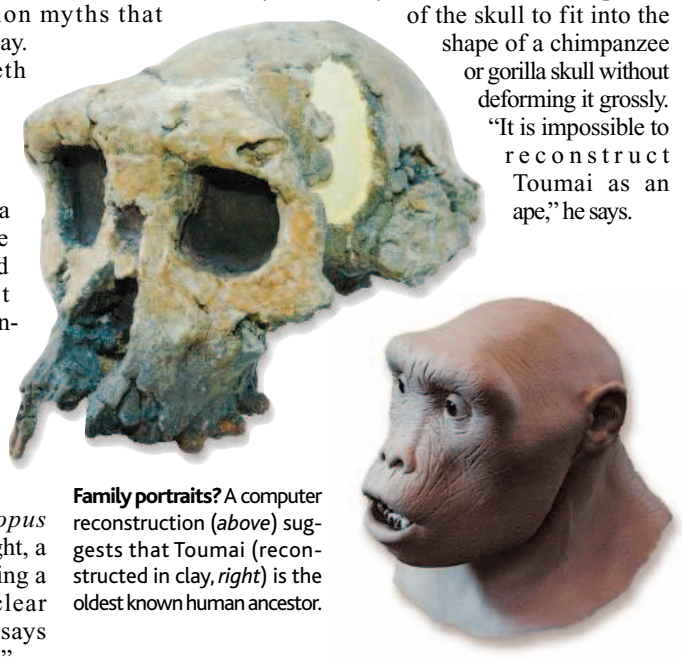
SOURCE (LEFT): THE SIGMA XI POSTDOC SURVEY; (RIGHT) © ROYALTY-FREE/CORBIS

PALEOANTHROPOLOGY

Facelift Supports Skull's Status as Oldest Member of the Human Family

For paleoanthropologists seeking the roots of humanity, a striking skull discovered among the shifting sand dunes of the Djurab Desert of Chad in 2001 was a dramatic find, offering the first glimpse of a primate alive at the dawn of humankind. But although the nearly 7-million-year-old skull was introduced as that of the oldest known hominid, rivals soon argued that it looked more like a gorilla ancestor than a human (*Science*, 12 July 2002, p. 171). Now the skull of *Sahelanthropus tchadensis*, nicknamed Toumai, is back in headlines again. It appears in *Nature* this week with two new looks—a three-dimensional virtual reconstruction and a clay bust on the cover, a nod to creation myths that humans were made of clay.

Fresh fossils of teeth and jaw fragments plus a state-of-the-art analysis of the virtual skull show that Toumai is indeed a hominid, or a member of the lineage that includes humans and our ancestors but not other apes, argues paleontologist Michel Brunet of the University of Poitiers, France, leader of the team that discovered Toumai. The new analysis also suggests that *Sahelanthropus* might have walked upright, a traditional marker of being a hominid. "It is quite clear Toumai is a hominid," says Brunet. "It is not a gorilla."



Family portraits? A computer reconstruction (*above*) suggests that Toumai (reconstructed in clay, *right*) is the oldest known human ancestor.

CREDITS: M.P.F.T. (ARTIST'S RECONSTRUCTION); E. DAYNES)

Other researchers applaud the sophistication of the reconstruction, performed by a team led by neurobiologist Christoph Zollikofer of the University of Zurich (UZ), Switzerland. "What a facelift! This beautiful reconstruction is the outcome of high technology combined with a deep understanding of anatomy," says Tel Aviv University paleoanthropologist Yoel Rak. But some caution that although the new evidence helps build the case that Toumai was a hominid, its identity is far from certain. "I'd be happy to put it down as [a very early] hominid," says anatomist Fred Spoor of University College London. "But it's a time we know so little about that I am still skeptical."

Brunet took the skull to Zollikofer and UZ anthropologist Marcia Ponce de Leon, known for their sophisticated high-resolution computed tomography scans and analyses. The skull had been crushed under a sand dune and distorted, and the researchers were able to erase the ravages of time in the computer, using three-dimensional computer graphics tools to rebuild it piece by piece. The resulting face is taller, with a bit more snout than seen in the original.

Zollikofer and Ponce de Leon then identified 39 landmarks on the skull, which they used to compare it directly with the skulls of fossil hominids, two chimpanzee species, and gorillas. They found that the shape of Toumai's skull "falls exactly within the hominids," says Zollikofer. No matter how they tried, they could not force the pieces of the skull to fit into the shape of a chimpanzee or gorilla skull without deforming it grossly. "It is impossible to reconstruct Toumai as an ape," he says.

Several researchers find the virtual evidence compelling. "I was worried about the distortion, but they are great at building virtual reconstructions that test hypotheses about how these fossils looked," says anthropologist John Kappelman of the University of Texas, Austin.

The reconstruction also revealed new evidence that suggests *Sahelanthropus* walked upright. A virtual line from the top to the bottom of Toumai's eye orbit makes roughly a right angle with another virtual plane at the base of the skull. That right angle is also seen in humans, reflecting that the head sits directly atop a vertical spine when walking upright. The angle between the planes is much smaller in the quadrupedal apes studied, reflecting that the head sits in front of a more horizontal ▶

ScienceScope

Hubble Relief

Finally, some good news for the Hubble Space Telescope. NASA engineers say that they can run Hubble on two gyroscopes rather than the three now operating. Space agency managers hope that turning off one gyro could extend Hubble's life by 6 months or more without affecting the quality of science returned. That could mean more time to revisit Hubble—either by shuttle or by robot—for an overhaul. Science chief Al Diaz says he will decide soon whether to turn off a gyro; currently, no repair visit is on the books, and the telescope is expected to die in late 2007 or early 2008.

NASA also says there is good news on the robotic servicing front. Engineers told *Science* that they have a plan to install two sets of three gyroscopes within an instrument now waiting on Earth to be installed in Hubble. With new gyros and new batteries, they say, Hubble could continue to operate for well over a decade. But incoming Administrator Michael Griffin likely will revisit the servicing issue. Griffin's Senate confirmation hearing is slated for 12 April.

–ANDREW LAWLER

Bay State Passes Stem Cell Bill

Massachusetts legislators overwhelmingly passed measures last week that explicitly allow research cloning, or somatic cell nuclear transfer (SCNT). The action promises to "put the state firmly in support of SCNT and other embryonic stem cell research," says Kevin Casey, director of government relations at Harvard University.

The state House and Senate have yet to agree on specifics of the final measure, which also would outlaw reproductive cloning. Republican Governor Mitt Romney opposes research cloning, but the bills passed by well over the two-thirds majority needed to override his promised veto. Senate president Robert Travaglini (D) has indicated that another bill is in the works that would earmark as much as $100 million to fund the research.

Harvard stem cell researcher George Daley is thrilled about what he calls "a real victory for science." Efforts to inform legislators helped, says Daley, who demonstrated nuclear transfer to a state senator. "I think this made it quite clear to him that SCNT is not about cloning babies," he says.

–CONSTANCE HOLDEN

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neck, explains co-author Daniel Lieberman of Harvard University. Thus the team concludes that *Sahelanthropus* "might" have been bipedal. "I'm the first to say you need postcranial fossils to be 100% sure, but it's darned hard to think how Toumai could not have walked upright," says Lieberman.

However, others caution that skulls don't walk upright by themselves, and that lower limbs are needed to prove this hallmark trait. Until Brunet and his colleagues describe postcranial fossils, paleoanthropologist Milford Wolpoff of the University of Michigan, Ann Arbor, sees Toumai as an ape, citing what he calls apelike features in the base of the neck.

More fossils also are needed to settle the question of how *Sahelanthropus* is related to later hominids. "There is still insufficient fossil evidence to determine whether there were one, two, or more hominid species lineages between 5 [million] and 7 million years ago in Africa," says paleoanthropologist Tim White of the University of California, Berkeley.

Brunet declines to comment on reports that his team has also discovered a partial thighbone, but he adds cryptically: "Surely postcranials will be coming in the future. I will be very, very surprised if it is not bipedal." –ANN GIBBONS

PALEOCLIMATOLOGY

Cosmic Dust Supports a Snowball Earth

Answering questions about Earth's climate of more than half a billion years ago can be a challenge—even questions as stark as whether land and sea were completely coated by ice from pole to pole. Indeed, the revival of the Snowball Earth hypothesis almost 7 years ago has bogged down of late, as paleoclimatologists have failed to turn up unequivocal evidence that ice enrobed our planet.

But on page 239 of this issue, a group of geochemists offers a new snowball marker: the element iridium, which continually rains down on us from space. They say they found so much iridium deposited at the end of a glaciation 635 million years ago that the planet must have been frozen pretty much solid for 12 million years straight. "I think this is a very exciting discovery," says geochemist Frank Kyte of the University of California, Los Angeles. Like any new tool, iridium needs some more work, but "I'm sure it will invoke a lot of discussion."

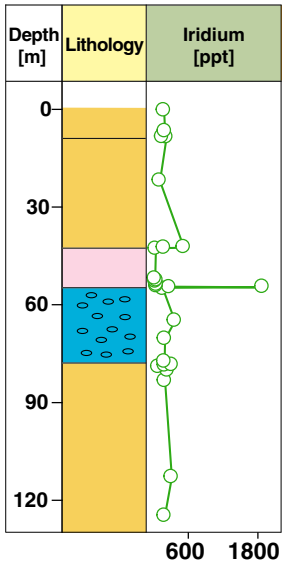
This isn't iridium's first appearance as a timekeeper. But geochemists Bernd Bodiselitsch and Christian Koeberl of the University of Vienna, Austria, and their colleagues took a new tack when they analyzed 44 elements including iridium along three cores drilled by copper miners in Zambia and the Democratic Republic of the Congo. Bodiselitsch and his colleagues figured that on an iced-over world, the iridium-rich meteoritic dust that rains onto Earth would accumulate until the snowball ended in a sudden meltdown, as climate modelers believe would happen. All the iridium accumulated in the ice would then be deposited in a single, thin layer of marine sediment. The more iridium deposited at the end of a snowball, the longer the snowball had gone on.

In the first few centimeters of sediment laid down on top of glacial sediments, Bodiselitsch and colleagues indeed found sharp spikes in the abundance of iridium. A spike showed up in all three cores at the end of the Marinoan glaciation about 635 million years ago and in two cores at the end of the earlier Sturtian glaciation about 710 million years ago. The iridium could conceivably have been homegrown—from a volcanic eruption or concentrated from crustal rock by some geochemical process—but several other elements were present in proportions typical of meteorites, not the crust. And the proportion of iridium to some other elements suggested that geochemical processing had not concentrated the iridium, they concluded. If meteoritic material was falling to Earth 635 million years ago at anything like the rate it has during the past 80 million years, the group calculates, the Marinoan glaciation lasted 12 million years, give or take 3 million years.

If the Marinoan ice age managed to save up 12 million years' worth of extraterrestrial iridium, it must have iced over the entire planet, researchers agree. The alternative to Snowball Earth has been Slushball Earth (*Science*, 26 May 2000, p. 1316). Rather than pole-to-pole ice, some paleoclimate modelers have suggested that Marinoan glaciation might have left tropical oceans ice-free and still produced glacial deposits near equatorial continents. But a slushball would have melted down within something like a million years as volcanoes belching carbon dioxide fueled a growing greenhouse. "It's hard to see what would keep a slushball around for 10 [million] or 20 million years," says climate modeler Raymond Pierrehumbert of the University of Chicago. And even if a slushball did last, its glaciers—unlike those of a snowball—would continually flow down to the sea, steadily depositing iridium, not producing a spike of it.

Geochemists are excited but naturally cautious. "Iridium is a strong indicator of extraterrestrial material," says Bernhard Peucker-Ehrenbrink of the Woods Hole Oceanographic Institution in Massachusetts. "However, it is just one of a series of useful tracers." He and others, he expects, will be pursuing other extraterrestrial tracers such as isotopes of helium and of osmium to test the claim of a Snowball Earth. Prompting such testing "is what good, interesting, provocative papers should do," he says. –RICHARD A. KERR

The ice was all around? A true Snowball Earth would have coated the globe with ice.



No accident. The discovery of a spike of cosmic iridium (green line) at the end of an ancient ice age (top of blue glacial sediments) suggests that ice covered the planet.

CREDITS (TOP TO BOTTOM): PHOTOS.COM; ADAPTED FROM BERND BODISELITSCH *ET AL.*

Now that international sanctions on Libya have been lifted, Muammar Gaddafi is inviting outsiders to help create a "northern star" of science and technology in Africa

From Pariah to Science Powerhouse?

TRIPOLI—When Mustafa Eteer, a doctor born in Libya, returned from Canada to visit his mother in this breezy Mediterranean city 4 years ago, he went through a life-changing experience. To his shock and dismay, his mother, who had been hospitalized for a minor illness, died of infections that would have been easily prevented in the West. The public health failure was "not a question of money," says Eteer. Oil revenue has provided Libyans the highest per capita income on the continent, even during the 12 years when the country was under international sanctions for its support of terrorism. Rather, he says, the isolation led to "a lack of knowledge and expertise," which in turn resulted in unnecessary suffering. Eteer vowed to bring modern medical science to Libya—although at the time, he had no idea how he would do it.

Today, thanks to a new drive to boost science by the nation's longtime ruler Colonel Muammar Gaddafi and the lifting of the last of the sanctions in 2004, Eteer may have a chance to realize his dream. He is overseeing the construction of a $100 million medical science complex on the outskirts of Tripoli that is being held up as an example of the nation's commitment to knowledge. The aim of the Center for Infectious Disease Control in Africa (CIDCA) is grander than Eteer's initial vision: Gaddafi wants it to tackle disease not just in Libya but throughout Africa. However, a dark shadow still hangs over this project and Libya's other scientific aspirations: With the government's acquiescence, a Libyan court has sentenced to death a group of foreign medics on conspiracy charges that outside experts say are based on bogus science (see sidebar on p. 184). The case could be a big impediment to enticing foreign scientists to work here.

The timing could not be worse for Libya's ambitions. As it seeks to dispel old ghosts, the country has embraced a goal of becoming a nexus of scientific and technical collaboration. CIDCA is just the first tangible feature of this vision. Gaddafi began setting the change in motion when he announced his abandonment of weapons of mass destruction (see p. 185) and agreed to pay billions of dollars in compensation for alleged terrorist attacks. Now, to the delight of Libya's academic community, Gaddafi is trying to position Libya as a leading light for the rest of Africa, in part by providing centers of scientific and educational excellence, including a new observatory for astronomers. Bankrolling these ventures is the Gaddafi Foundation, a huge private fund of undisclosed value created by the Libyan ruler and directed by his son Saif.

"The sanctions killed Libyan science," says Ali Al-Hamdy, an ecologist who directed his country's Marine Biology Center until last year. "It was nearly impossible to go to conferences, publish in journals, or get equipment. But now, everything is possible."

Elite site. The campus of Libya's Center for Infectious Disease Control in Africa boasts tennis courts and irrigated landscaping.

A CDC for Africa

Beneath the vaulted arcades of the European-styled boutiques in Tripoli's Green Square, immigrant workers sweep up trash in preparation for a state ceremony. Most laborers like these are here illegally, having crossed the dangerous expanse of the Sahara to escape violence and poverty in Sudan, Chad, Niger, and farther south. With them comes the specter of diseases that Libya has until now largely escaped, such as AIDS. Libya's new focus on pan-African diseases is therefore built in part on enlightened self-interest.

"The Libyans usually say they have no AIDS," says Vittorio Colizzi, a molecular pathologist at Tor Vergata University in Rome who has collaborated for several years with Libyan epidemiologists, "but it has definitely been here for years and is increasing." Others agree that the risk is real. "All of northern Africa has the right ingredients for an AIDS epidemic, including poverty, civil wars, and large refugee populations," says Mark Kline, a virologist who directs the International Pediatric AIDS Initiative at Baylor College of Medicine in Houston, Texas. There's no reason to think Libya will be exempt. But gauging the problem is difficult, he says, because the region falls into "a real hole in terms of epidemiological data."

Colizzi and Massimo Amicosante, a biologist also at Tor Vergata, are here to start plugging that hole. They are meeting with Eteer to finalize an exchange program that will get Italian and Libyan disease researchers working together. The Italian government is an eager partner: It hopes for better disease prevention among the tens of thousands of sub-Saharan refugees each year who use Libya as the departure point on their way to Europe.

Unruffled by Tripoli's fierce traffic, Eteer offers to take the Italian researchers—and *Science*—on a tour of the planned CIDCA site, beginning with a drive through the city's scrubby and desolate outskirts. After arriving, we slip through a gate in the barbed-wire-crested wall that surrounds the CIDCA compound and enter a different world. With the help of constantly running sprinklers, lush vegetation sprouts between the tennis courts, guesthouses, and other white-washed buildings. Eteer hopes such amenities, all paid from the deep pockets of the Gaddafi Foundation, will help entice Western disease researchers to work here.

In the main administration building, offices are filled with plastic-wrapped chairs and tables like gifts waiting to be opened. "I want the best scientists in the world to come work here, side by side with Libyans," says Eteer, with a doctor's reassuring smile. Drawing comparisons with the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, he envisions Tripoli as the focal point of a collaborative network. But at least initially, the scope of the Libyan institute will be narrower: It will focus on Africa's three big killers, namely AIDS, malaria, and tuberculosis.

CREDIT: J. BOHANNON/*SCIENCE*

No mirage. Gaddafi appears as hero in his own "Epic of the desert," a poster about a massive water project to supply coastal cities.

Although he does not expect the last bulldozer to leave the site until 2007, Eteer is already seeking a research director. Initially, he says, there will be room for 100 scientists doing a mixture of basic molecular research and epidemiology. To generate steady income, a diagnostic laboratory will handle all of Libya's blood samples, which are currently shipped abroad for testing, amounting to $10 million of potential income annually. There are also plans to build a factory on site to produce generic drugs for the three diseases. Competition for positions at CIDCA will be "open, like any Western institute," but Eteer adds that the emphasis will be on building collaborations with Africans who "stand to gain the most" from Western expertise.

"It is a grand plan, and badly needed," says Bashir Allaghi, one of Gaddafi's personal physicians, who sits on the board of the Gaddafi Foundation. "We Libyans are open to the world. We are hungry for contact."

Outsiders seem optimistic but wary. The center could be a windfall for "the massive shortage of expertise in Africa," says Kevin de Cock, director of CDC's Kenya field station. But without "very careful diplomacy," he warns, it could also be a flop. One potential pitfall is its location. "Libya is far from sub-Saharan Africa, where these diseases are at their worst, so they will have to build satellite centers for fieldwork," he says. And Gaddafi's pronouncements that Libya "speaks for Africa" have caused tension. De Cock is surprised to have heard about CIDCA only recently. "Are they going to collaborate or compete?" he wonders.

Perhaps the biggest surprise is that "executive control" of CIDCA will be handed over to a Westerner. "I don't want a Libyan to be in charge," says Eteer. "Only an outsider can be free from all the political pressures here." Allaghi says the Gaddafi Foundation backs the plan. But one skeptic is Colizzi. "Will the Libyans really put a $100 million facility in Western hands? No way." Another question is who would be willing to take up the post if Libya carries out the death sentence it imposed last year on a group of foreign medics. The opinions of many likely candidates, such as Hans Wigzell, director of medical research at the Karolinska Institute in Stockholm, Sweden, who calls the medics "Libya's scapegoats," are already hardening.

Prodigal son. Mustafa Eteer returned from Canada to help build Libya's disease research center.

In spite of such doubts, Colizzi believes Libya's ambitions to bring modern medical science to Africa are genuine. "They do want to make this work," he says. "The question is if they will really let science be independent of politics."

Getting started

CIDCA is a sign of things to come for Libya's academic community, according to Abdusalam Al-Gallali, Libya's new secretary of higher education. "We now have all the money we could need," he says, "and our priority is to improve the quality of work and teaching."

Libya now claims to have set a high standard for the rest of the Arab world. Unusual for a Muslim nation, there appears to be gender equality in higher education: 50% of students are female, according to Al-Gallali, and the highest academic position—chancellor of El-Fateh University in Tripoli—is occupied by a woman. He also claims that Libya is second only to Canada in per capita attendance at university, where postsecondary education is estimated to be just over 50%.

Libya hopes to employ these highly trained young people and keep them from emigrating, says Yusef Mabosut, a U.S.-trained microbiologist who was chancellor of El-Fateh until 2000. For some fields, such as geology and engineering, there seems to be endless room for growth. Beyond the oil industry, Gaddafi's $28 billion "Great Man-

CREDITS: J. BOHANNON/*SCIENCE*

made River Project," called the largest public works project in the world by the United Nations Educational, Scientific, and Cultural Organization, has employed thousands to pipe water from deep beneath the Sahara to Libya's coastal cities—and it's far from finished. But for students in other fields, the prospects look grim. This is one reason why the government is ramping up funding for new scientific projects such as CIDCA. Among others to benefit are the country's small community of astronomers; they are getting a new observatory.

Last year Gaddafi personally contacted the French electronics company Sagem to buy a $13 million telescope, making up for a lost purchase from Germany that fell through during the embargo. Gaddafi is "passionate" about astronomy, says François Querci, an astrophysicist at the Observatoire Midi-Pyrénées in Toulouse, France. Plans call for the telescope to be set up in Libya's southeastern deserts, although abundant landmines—a legacy of war with neighboring Chad—could pose a problem. Querci was there in February to meet with the country's top astronomer, Hadi Gashut, to discuss Libyan participation in an embryonic network of observatories in Muslim countries. "Libya is taking a leadership role," says Querci.

With a 2-meter-diameter mirror, the Libyan telescope will be modest by world standards, but it will be perfect for studying variable stars that require continuous observation, says Michael Bode, an astrophysicist at Liverpool John Moores University in the United Kingdom. It will also be valuable, says Querci, as the only view from northern Africa, where the skies are far clearer than those over Europe along the same longitude. Observations are expected to begin in early 2006.

Meanwhile, for Libyan archaeology the lifting of the embargo looks like a mixed blessing. The government, anticipating a boom in tourism, has renewed interest in researching and preserving the region's 10,000 years of human settlement. That should translate to more archaeology jobs, says Sa'ad Abdul Aziz, director of the Germa Museum in southern Libya, who coordinates archaeological research for Libya's Department of Antiquities. But at the same time, development of oil exploration, roads, and pipelines also may skyrocket. And particularly for the most vulnerable and least studied of Libya's archaeological heritage—such as the ancient rock engravings that can be found on boulders throughout the southern deserts—this spells trouble. With so much of Libya's income dependent on petroleum, it seems unlikely that archaeology will be given higher priority.

But Aziz is optimistic. "We have been very isolated," he says, "but that time is over. Everyone just wants to start the work."

—JOHN BOHANNON

John Bohannon is a science writer based in Berlin.

Evidence Overruled: Medics on Death Row

TRIPOLI—The toll of the past few years shows on Zdravko Georgiev's ashen face. He was working as a doctor in southern Libya in 1999 when he heard that his wife Kristiyana—a nurse in the northern town of Benghazi and, like him, a Bulgarian—had been arrested. He rushed back to Benghazi and immediately contacted the police for information. After several days without word of his wife, he was arrested, too. And then, he says, the nightmare began: Torture without explanation, police interrogations, and accusations of conspiracy.

End of the line. Six foreign medical workers in Libya have been sentenced to death on improbable charges of bioconspiracy; a final decision is pending.

CREDIT: GEORGI MILKOV

After holding them for a year without access to lawyers or the outside world, prosecutors charged Georgiev, his wife, three other Bulgarian nurses, and a Palestinian doctor at the Benghazi hospital with acts of bioterrorism. The Libyan government accused the foreign medics of deliberately infecting children under their care with a strain of genetically engineered HIV. By then, hundreds of children at Benghazi's Al-Fateh hospital were found to have been mysteriously infected with HIV, and many had already died of AIDS-related illnesses. Today, the medics deny any wrongdoing, although they earlier gave confessions which they—and doctors who have been allowed to examine them—say were extracted under torture.

Protests from abroad prompted Libya to invite European scientists to come to Benghazi and study the outbreak. The team, led by Vittorio Colizzi, a molecular pathologist at Tor Vergata University in Rome, and Luc Montagnier, a virologist at the Pasteur Institute in Paris and co-discoverer of HIV, concluded that the evidence was overwhelmingly in favor of the medics' innocence. They put the blame for the infections on negligent practices hospital-wide. But this opinion was rejected. To the shock of international observers, the Libyan court found the medics guilty last year and sentenced them to death by firing squad. Libya's Supreme Court will announce its verdict on the medics' final appeal on 31 May. Gaddafi has said he believes they are guilty.

Western observers condemn the Libyan proceedings. "This was a betrayal to my profession," fumes Hani Shennib, a Libya-born professor of surgery at McGill University in Montreal, Canada, who is coordinating relief efforts for the infected children.

The timing could not be worse for Libya's efforts to shake its reputation as a lawless pariah state. The future of at least one major new Libyan science initiative—a disease research center near Tripoli—hangs on the outcome of this case (see main text). Outsiders see it as a test of whether Libya is truly ready to host such an institution. "Who would want to come work in a country where science and logic are not respected?" asks Hans Wigzell, director of medical research at the Karolinska Institute in Stockholm, Sweden. Wigzell was one of the authors of an open letter last year,

Agencies Plan Exchange With Libya's Former Weaponeers

U.S. officials are trying to involve Libya's weapons experts in outside collaborative projects. One potential hitch: The Libyans want to keep working on missiles

After months of delicate planning, the United States is embarking on a groundbreaking effort to bring Libya's former weapons researchers in from the cold. The initiative is expected to include exchange programs to foster scientific and commercial cooperation between Western and Libyan scientists and a "sister" link between Lawrence Livermore National Laboratory in Livermore, California, and the Tajoura Nuclear Research Centre in Tripoli, Libya, which had been the nerve center of Libya's nuclear weapons program.

Physical evidence. A U.S. guard in Oak Ridge, Tennessee, stands watch over contraband nuclear materials Libya surrendered last year.

"The steps being taken to engage the [former weapons] scientists are an important make-or-break element of Libya's reattachment to the international community," says Rose Gottemoeller, an expert on nuclear nonproliferation at the Carnegie Endowment for International Peace in Washington, D.C. "If key elites such as the scientists" don't participate, she warns, "the danger of backsliding and failure becomes strong." Building an esprit de corps with Libyan researchers could have a broader political payoff as well: It "can only be seen as a good deed in a time that most Muslim countries don't look too kindly on us," says Jack Boureston, managing director of First-Watch International, a nonproliferation think tank in Monterey, California.

In contrast to the U.S. government's spotlighting of recent initiatives to find civilian work for former weaponeers in Iraq, few details about the Libya effort have been released. The U.S. State Department has asked U.S. officials and scientists in the U.S. national laboratories not to speak with the press about the Libya initiative; nonetheless, several agreed to speak with *Science*, but only on condition of anonymity. U.K. officials also involved in the effort declined to comment.

This caution, *Science* has learned, derives from a concern about ongoing, intricate negotiations with segments of Libya's former weapons community. Libyan officials apparently have asked that the evolving initiative not yet be publicized. "One particular concern" from the U.S. side, says a senior Bush Administration official, is that Libya's ballistic missile

signed by 28 scientists from eight countries, calling on Gaddafi to recognize the scientific evidence and intervene on the medics' behalf.

It seems as if "science itself has been on trial here, and lost," says Colizzi, who was called as an expert witness by the Libyan court. Colizzi and Montagnier studied the outbreak in Benghazi between 2002 and 2003. "We were supposed to have free access to all the materials and data to make an objective study," says Colizzi, "but in fact hospital officials tried to block us at every step."

The first surprise came when they asked to see the "smoking gun" in the case, a pair of vials allegedly found in the home of one of the Bulgarian nurses. In the court, a Libyan doctor presented the vials along with the results of a Western blot—a test using antibodies to detect the presence of a particular protein—which he said "proved" that the HIV outbreak originated from stocks kept secretly by the medics. Upon seeing the blot, Montagnier says, he concluded "it just looked like background noise." He proposed to test the vials with the more sensitive polymerase chain reaction to look for specific RNA sequences of the virus. But officials never made the vials available.

In spite of such hindrances, Colizzi and Montagnier were able to obtain blood samples and medical records from the children, examine the hospital, and interview its staff. It soon became apparent, says Colizzi, that "this is a classic nosocomial infection" in which tainted blood is accidentally passed between patients through poor hygiene practices, such as the reuse of disposable syringes and catheters, insufficient sterilization of instruments, and a general lack of quarantine between patients. The most compelling evidence of contamination they found, according to Colizzi, was the presence of other viruses in the children's blood, including several strains of hepatitis C. "This is extremely unusual for children," says Colizzi, "but is easily explained if there have been multiple accidental contaminations in the hospital."

Colizzi and Montagnier maintain that these results alone should be enough to exonerate the accused, but Colizzi thinks another piece of evidence puts their innocence "beyond doubt." According to the hospital medical records, some of the children became infected with HIV before the medics even started working at the hospital. And in one case, a child of HIV-negative parents became infected at birth in the hospital, long after the medics had been arrested. According to the prosecution, the dates are errors in the hospital's record keeping.

The prosecutors have had some difficulty, however, sustaining the charge that the strain of HIV was engineered in a lab. "They jumped to this conclusion," says Colizzi, "because this particular HIV strain did not appear in GenBank," the database of genetic sequences maintained by the U.S. National Institutes of Health in Bethesda, Maryland. The Libyan government backed away from the claim in 2002, referring to the case as one of homicide rather than bioterrorism.

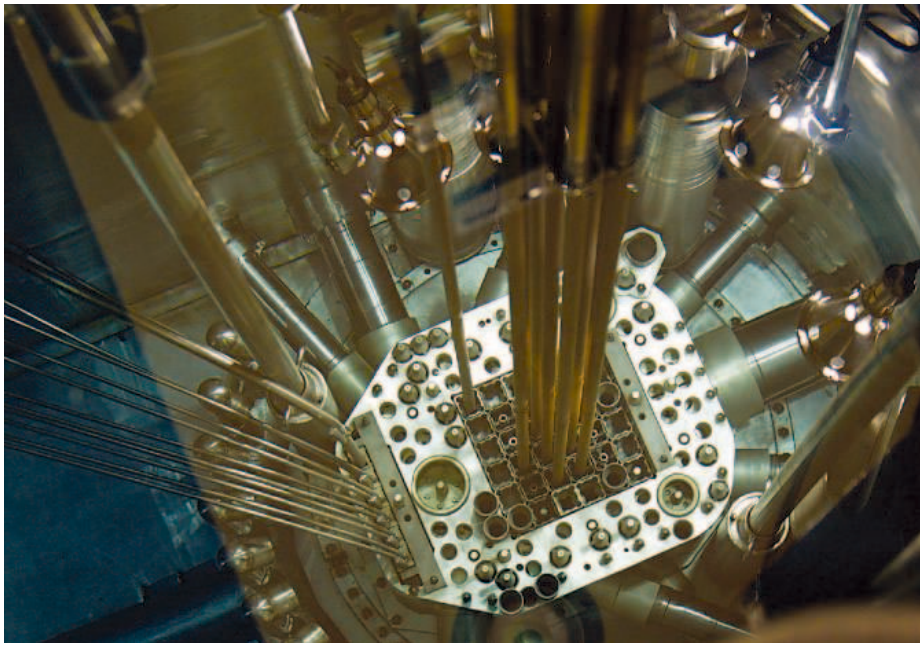
In 2003, in the presence of international observers, including ambassadors from 13 countries, Colizzi and Montagnier presented their findings to the Libyan court. Five Libyan doctors at the same time accused the Europeans of being "unscientific" because of inconsistencies in some of the clinical data. Colizzi responds that the source of the data was the hospital itself, and that if the disputed data are removed, the case against the Bulgarians still doesn't hold up. But the Libyan judges were not persuaded: In May 2004, they declared the medics guilty. Georgiev alone was released with a suspended sentence, perhaps in light of the fact that he had not even been working at the hospital. He now lives within the protection of the Bulgarian embassy in Tripoli, where he met with *Science*.

Since the sentencing, the Libyan government has said that the case might be "reconsidered" if compensation is paid—a sum of more than $5.7 billion was suggested—and if the British government is willing to release a Libyan accused of the 1988 bombing of a jet over Lockerbie, Scotland. "Scientific thinking has no more role to play in this anymore," says Colizzi. "It's completely political now. Or perhaps it always was."

–J.B.

CREDIT: REUTERS/PAUL EFIRD/*KNOXVILLE NEWS SENTINEL*

researchers "want to keep working on missiles. Obviously that's a problem," he says. According to a nonproliferation official at the U.S. Department of Energy (DOE), the Libyan government claims to have 4000 missile scientists and technicians, including 500 with advanced degrees—the largest subset of former weaponeers being courted for exchanges.



Hot source. A research reactor at the Tajoura nuclear facility was used to make weapons-grade uranium.

Libya's rapprochement with the West began after it announced on 19 December 2003 that it would disband its R&D on nonconventional weapons and eliminate existing stockpiles. Since then, investigators from the U.K. and U.S. governments and the International Atomic Energy Agency (IAEA) in Vienna, Austria, have assembled an increasingly detailed picture of the once-clandestine programs.

Libya's nuclear R&D effort was more ambitious than suspected, but analysts believe it was at least several years away from a bomb. An IAEA report last year noted that in the 1980s uranium targets were irradiated in Tajoura's Soviet-made, 10-megawatt research reactor. Tiny quantities of plutonium were separated from at least two targets at a radiochemical laboratory, although investigators now believe that Libya focused solely on a uranium bomb. In March 2004, the IAEA report notes, about 13 kilograms of fissile uranium-235 were flown from Tajoura, on the eastern edge of Tripoli, to Russia for blending into power plant fuel. When it came clean in late 2003, Libya revealed a further 11 nuclear sites and 15 weapons-related sites.

One particularly disturbing facet, experts say, is that Libya readily obtained materiel and know-how through a global nuclear black market, primarily the network run by the father of Pakistan's bomb, Abdul Qadeer Khan. "In our estimation there was not a lot of indigenous talent. [Libya] procured the equipment and bought the recipes," says the Administration official. The IAEA report recounts how in late 2000 Libya had outfitted a pilot enrichment facility with three cascades of centrifuges based on an advanced design from Pakistan, before mothballing the equipment 2 years later for security reasons. In January 2004, a U.S.-U.K. team shipped 25,000 kilograms of Libya's most sensitive items, including centrifuge parts and uranium hexafluoride—the gas fed into centrifuges for concentration—as well as weapons designs to Oak Ridge, Tennessee, for analysis and destruction.

According to Libya, 800 nuclear specialists, including 140 with advanced degrees, were involved in the program. "Right now we have no way to vet those numbers," says the Administration official. U.S. officials note that some senior Libyan weaponeers were educated in Europe, particularly the United Kingdom, or in the United States before sanctions were imposed on Libya in the 1980s.

Although Libya wasn't close to going nuclear, it produced chemical weapons and was accused by Chad of using mustard gas against its forces during a border conflict in 1987. (Libya has denied the allegation.) In March 2004, Libyan officials declared to international investigators that the country had stockpiled 23 tons of mustard gas at its al-Rabta facility, which Italy is now helping convert to a pharmaceutical plant. Libya says it has 120 former chemical weapons workers but just 12 with advanced degrees, according to the DOE official.

"The Libyan nuclear and chemical scientific core is certainly smaller and less seasoned than that of other rogue regimes such as Iraq," says Sammy Salama, a Middle East expert at the Monterey Institute's Center for Nonproliferation Studies in California. However, he says, their "relevant firsthand experience"—operating the Tajoura reactor and cooking up chemical weapons—"makes Libyan scientists desirable for other rogue regimes that may have WMD aspirations."

Libya's ballistic missile program, meanwhile, was centered on relatively primitive Scud designs. "Their missile work isn't thought to be all that great," says a U.S. specialist. The Administration official insists that "carrots exist" to get researchers to abandon missile R&D; he declined to elaborate. Inspectors are still probing whether Libya had more than a passing interest in biological weapons.

The U.S. State Department has taken the lead in organizing workshops between U.S. and U.K. experts and Libyan counterparts. The first two meetings, last October and December in Tripoli, explored potential collaborations on a hydrological and geochemical database, an environmental monitoring lab, technologies for water purification, and the production of medical radioisotopes.

One initiative gaining traction is a sister lab agreement between Livermore and Tajoura. Although not finalized when *Science* went to press, the arrangement would resemble a successful DOE program for Russian nuclear scientists begun after the Soviet Union's dissolution in 1991. Joint research could include, for example, neutron activation analysis for materials science using Tajoura's research reactor and associated labs. DOE's National Nuclear Security Administration also aims to help convert the reactor from using highly enriched to low-enriched uranium fuel.

In the meantime, U.S. officials are organizing a reciprocal visit later this spring of a Libyan delegation. The itinerary would include Livermore and Sandia National Laboratories and sites where Cold War–era nuclear weapons facilities have been dismantled—possibly Hanford in Washington state and Savannah River in South Carolina.

Unlike the engagement effort in Iraq, U.S. officials expect oil-rich Libya to bring significant resources to the table. "They don't need our money; they need ideas," says the Administration official. "Their main priority is partnership." It's a fragile relationship that's just beginning to blossom.

–RICHARD STONE

CREDIT: JOHN MOORE/ASSOCIATED PRESS

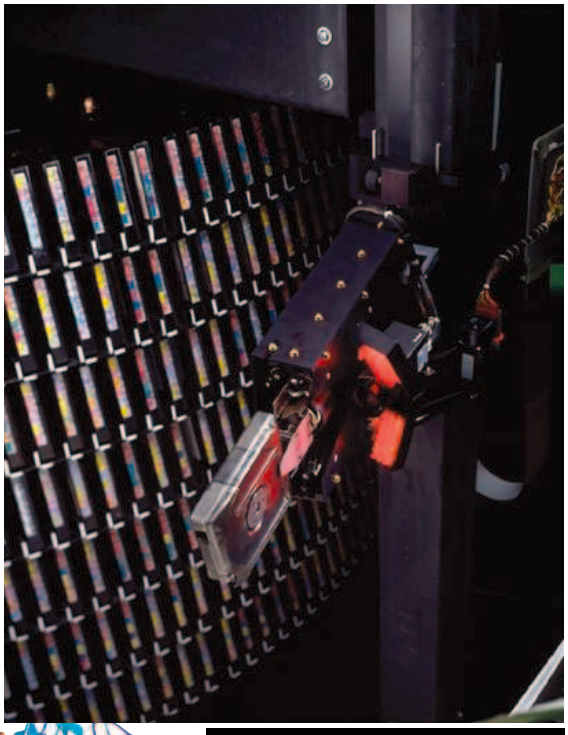
Boom in Digital Collections Makes a Muddle of Management

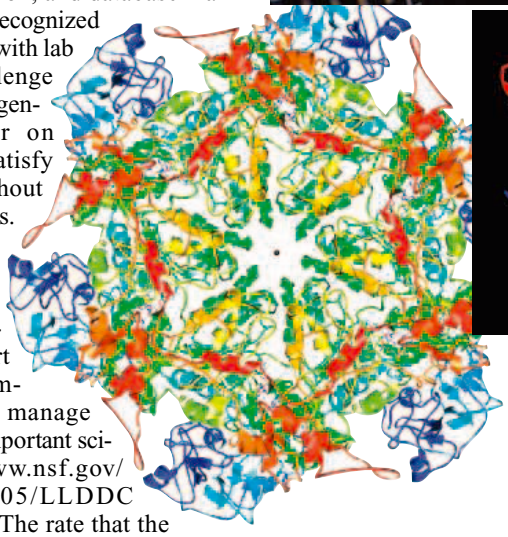
Electronic collections are a huge boon to scientists. But a new report says NSF needs to pay more attention to how they are funded and operated

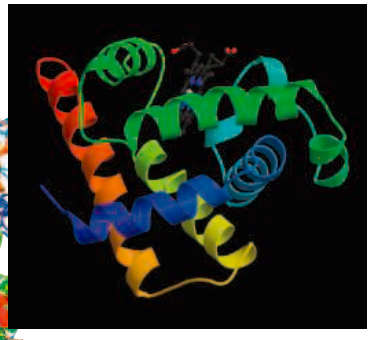
Forget trays of preserved insects with their informational tags, as well as collections of rocks, fossils, and other samples from nature's treasure chest. Data have gone digital, and researchers from all walks of science—from climate modelers to systematists—are stockpiling their observations in newly created databases accessible to everyone through the World Wide Web. But as researchers head full speed into the digital world, the U.S. National Science Foundation (NSF) wants to ensure that they don't run out of gas along the digital highway and that the rules of the road are clear to everyone.

To date, NSF has not been tracking its total commitment to the increasing number of digital data collections. Yet once started, these collections require continued—and likely increasing—support. At issue too are policing data to maintain standards of data quality, formatting data for eventual incorporation into metacollections, and presenting the information in ever-more-sophisticated, yet understandable, displays. More students and researchers need to know how to use the information, and database management should be recognized as a career on a par with lab research. The challenge for NSF and other agencies (see sidebar on p. 189) is how to satisfy all these needs without busting their budgets.

Last week, NSF's oversight body, the National Science Board (NSB), approved a draft report that calls for a comprehensive plan to manage this increasingly important scientific asset (www.nsf.gov/nsb/meetings/2005/LLDDC_Comments.pdf). "The rate that the data are increasing is exponential," says board member Michael Rossmann, a structural biologist at Purdue University in West Lafayette, Indiana. Adds Anita Jones, a computer scientist at the University of Virginia in Charlottesville and former board member, "I am concerned about the growing bill." The board is eager for community input.







Data boom. As with other data, molecular structures have gone from simple (*right*) to complex (*left*) and require sophisticated storage technologies (*above*).

CREDITS (TOP TO BOTTOM): COURTESY OF STORAGETEK; B. DELABARRE, A. T. BRUNGER, *J. MOL. BIOL.* **347**, 437 (2005)

A growing concern

Digital databases date back to the era of punch cards and computer tapes. In the 1970s, crystallographers agreed to deposit their data in the newly created Protein Data Bank (PDB) at Brookhaven National Laboratory in Upton, New York. The bank is now managed by the Research Collaboratory for Structural Bioinformatics located at Rutgers University in New Brunswick, New Jersey, and the University of California, San Diego. Each week a staff of 25 adds 100 new molecular structures to the 30,000 already deposited. About 10,000 individuals visit the database daily, says its head Helen Berman, who calls PDB "the center of the new biology."

Such a growing enterprise requires continued funding. PDB's annual budget has grown 200-fold since 1976, to about $6 million. Some $2 million comes from NSF, and eight other organizations chip in the rest. "It's money well spent," says NSB Chair Warren Washington. "We cannot afford to have these data sets lost or poorly handled."

A climate modeler at the National Center for Atmospheric Research (NCAR) in Boulder, Colorado, Washington knows how valuable long-term data sets can be for simulations and other research efforts. NSF provides about two-thirds of NCAR's $139 million annual budget, but NSF's contribution to its dozens of databases is harder to quantify, says Richard Anthes, president of the University Corporation for Atmospheric Research, which oversees NCAR. "It is on the order of about $10 million," he estimates.

NCAR databases contain an estimated 1.6 petabytes of oceanographic, climate, and other information. The size of its Scientific Computing Division data-support section doubled last year, says its manager Steve Worley, who adds, "I imagine that's happening for almost everybody. I don't see any end." As with other databases, new entries need to be formatted and incorporated into the existing databases, which are updated regularly to take advantage of the latest storage technology.

These two projects illustrate the growing importance—and expense—of keeping data accessible, possibly in perpetuity, to all who want to use them. NSF doesn't have a good handle on its portfolio, says NSB executive officer Michael Crosby, who guesses that the agency could be supporting "hundreds, even thousands," of digital data collections. They range from those built to suit an individual researcher's needs to ones that are essential to many disciplines. The mode of funding is equally haphazard, says NSB member and ecologist Daniel Simberloff of the University of Tennessee,

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Canadian Report Calls for Data Agency

OTTAWA—Canada needs an agency dedicated to ensuring maximum access to the fruits of publicly funded research.

That's the conclusion of a task force formed by a bevy of scientific organizations, which last week urged government officials to create a national data preservation and management organization. Such an agency would craft a national strategy relating to the acquisition, maintenance, and dissemination of all types of research data, from published scientific material to electronic archives and databases. A blue-ribbon panel chaired by David Strong, president of the private University Canada West in Victoria, British Columbia, suggested that the Canada Foundation for Innovation, which helped back the 9-month study, take the first step by providing start-up money.

The initial questions to be examined include many of those addressed in a draft report from the oversight body of the U.S. National Science Foundation, such as standardization of format, training, and funding for databases (see main text). Proponents hope that federal legislators will create a statutory agency—called Data Canada—with a $2.5-million-a-year budget to investigate a "central data preservation and management facility and a series of access and service nodes located in research institutions" across the country. The panel didn't speculate on how much it would cost to create and operate such a system. **–WAYNE KONDRO**

Wayne Kondro is a freelance writer in Ottawa.

Knoxville. "What we are asking NSF to do is come up with a single strategy" for evaluating and prioritizing these projects, he says. Part of that strategy should include criteria to determine continued support. There should also be guidelines about the right balance between data maintained and the acquisition of new data, says Rossmann.

Projects such as PDB and the NCAR collection illustrate how a decision years ago to support a database can have significant, long-term implications for NSF's budget. "Clearly the current trend is to spend a large proportion [of NSF's database support] on maintaining databases," says Rossmann. When times are tight, however, that emphasis could mean fewer research awards.

Data-rich but poor

That doesn't mean database managers are feeling flush, however. "We have money troubles all the time," NCAR's Worley says, citing his desire to incorporate data from different collections into a single, seamless data resource. But that goal has taken a back seat to maintaining what's already on hand. Likewise, a compendium of *Arabidopsis* data at the Carnegie Institution Department of Plant Biology in Stanford, California, and the National Center for Genome Resources in Santa Fe, New Mexico, recently received about $3 million less from NSF than the almost $11 million its managers had requested for the next 5 years. "We ended up having to give up a lot of innovative stuff," says *Arabidopsis* Information Resource lead investigator Seung Yon Rhee, a Carnegie plant biologist.

One solution to funding shortfalls is to find other backers. Both NCAR and PDB supplement NSF's contribution with money from other federal agencies and international organizations. In other cases, host institutions are expected to cover costs for maintenance and upkeep. That's been the approach taken by NSF's Biological Research Collections program, which has helped keep natural history specimens in good shape but which now limits awards to one-time support of specific goals and projects.

To make NSF's money go further, biological research collections program manager Mark Farmer spends about half of his $4.5 million budget on a new long-term digital data collection—a "virtual" natural history museum with a portal that will provide desktop access to the world's preserved plants, animals, rocks, and so on. At the same time, museums and universities have agreed to bear the cost of operations for their collections, including keeping the links current and the original specimens in good shape. "We don't want to get into the business of paying for permanent staff at an institution," says Farmer.

CREDIT: PDB

Avoiding obsolescence. To be useful, digital databases require constant improvements to data storage, quality, and accessibility.

The science board's goal, says Simberloff, is "to make sure that the data collections we are funding are of the highest quality, that standards for storage and access are good." Toward that end, its report asks NSF to tally up all databases under its wing and to establish consistent rules to evaluate and fund them. That may include clarifying who is in charge of policing the data and requiring a database management plan covering the kind of data to be included, the standards for quality, and the criteria for what will be archived.

Key human resources issues also need to be addressed, says NSF program director Chris Greer. One big issue is encouraging database managers to develop new ways to disseminate the information more broadly. Greer cites the PDB's "Molecule of the Month," which provides online images and lay-language summaries of a protein's structure, function, and relevance to human health, as an excellent example of outreach to students.

A second issue is preparing undergraduates, graduate students, and postdoctoral fellows to take advantage of all these databases. NSF's 2006 budget request, now pending, includes a new program to expand competence in computing and other skills needed by 21st century scientists. Greer says that even more focused training programs may be needed.

Finally, Greer and others say that those who maintain these databases should be recognized as credible scientists whose work warrants tenure and other career advancements. "They are collectively an outstanding resource," Greer says. Toward that end, PDB's Berman says she encourages her employees to write research papers and speak at conferences and offers opportunities for career advancement. "It's very important to keep them motivated," she points out.

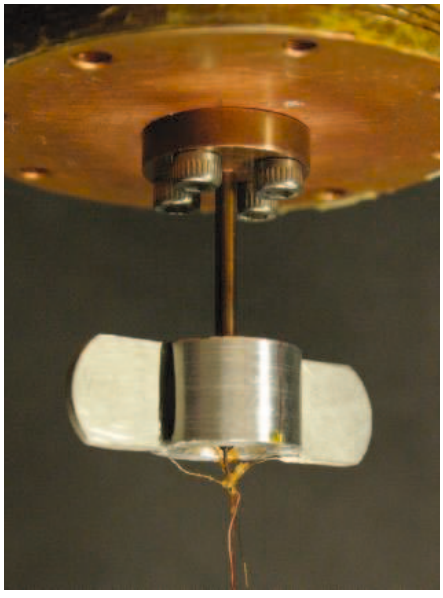
The science board's report sends NSF a signal that there's work to be done. "The NSF strategy and policies have not kept pace" with what's needed, the report points out. But Berman is optimistic that NSF will catch up. "The best thing is that NSF is now prepared to think about this."

–ELIZABETH PENNISI

Signs of a Second Flowing Solid Deepen a Quantum Mystery

Los Angeles, California—From 21 to 24 March, more than 6500 gathered to ponder flowing solids, splashless droplets, and living crystals.

If two's a trend, then bizarre flowing solids are la mode. In the past year, experimenter Moses Chan and colleagues at Pennsylvania State University, University Park, have reported that, at high pressures and temperatures approaching absolute zero, solid helium appears to flow like a liquid without any viscosity. Now, as theorists debate how such "superflow" is possible in a crystal, Chan and Penn State's Anthony Clark report that solid hydrogen seems to behave in the same strange way.



Twisted. Within this oscillating can, solid helium appears to flow with no resistance.

The preliminary hydrogen data met with skepticism from some researchers. "The temperature they're seeing [the onset of flow] at is right at the temperature they see it for helium," notes James Day, a physicist at the University of Alberta in Edmonton, Canada, and that coincidence could point to some undiscovered experimental artifact. "I think it's a little suspicious," Day says. But others argue that in key regards, hydrogen is similar to the helium isotope used in the earlier experiments, helium-4. "If it's seen in one, it should be seen in the other," says Milton Cole, a theorist at Penn State.

Researchers presented several theories to explain the helium results, but none of them accounts for every experimental detail. On one point everyone agrees: If the solid really does flow, "supersolid" helium would rank among the most important discoveries in low-temperature helium physics, a field that has already nabbed four Nobel prizes. "I expect it to get the Nobel," Cole says, "*if* it's right."

Since 1937, physicists have known that at temperatures below 2.17 K, liquid helium-4 can flow without resistance. That happens because many helium atoms collapse into a single quantum wave that resists disturbances. For decades theorists have speculated that a similar thing might happen in solid helium-4, too. The original idea was that missing atoms, or vacancies, within a helium crystal could team up to form a free-flowing fluid of their own, so that the superflow of missing atoms in the solid would mimic the superflow of real atoms in the liquid. But experimenters had not been able to produce the elusive supersolid.

To spot it, Chan and Eunseong Kim employed a torsional oscillator—essentially a little can of helium rotating back and forth on the end of a thin shaft. The can oscillates at a frequency determined by the stiffness of the shaft and the can's inertia, which in turn is determined by the mass of helium stuck to it. Chan and Kim pressurized the helium to between 25 and 145 times atmospheric pressure to ensure that it solidified. At each pressure, the frequency of oscillation climbed suddenly as the temperature dipped below 2 tenths of a kelvin. Those upswings suggest that some of the helium lets go of the oscillator and slips through the crystal without any resistance.

But just how that's possible remains a subject of controversy. Vacancies probably cannot account for the data, Chan explains, because if they are so mobile, they ought to wander to the edges of the crystal and blink out of existence. On the other hand, computer simulations by theorist Bryan Clark of the University of Illinois, Urbana-Champaign, indicate that in a flawless crystal of helium-4, the atoms cannot collapse into a single quantum state.

Boris Svistunov and colleagues at the University of Massachusetts, Amherst, theorize that solid helium consists of many small crystalline grains and that superflow occurs along the boundaries between them. But experimenters have evidence that helium forms large grains that would provide too little interfacial area to account for the observed flow.

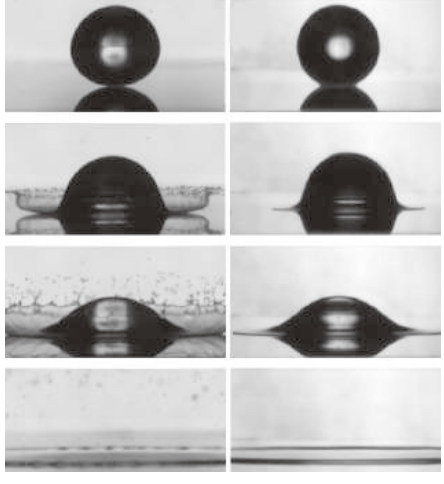
Ultimately, physicists will have to rethink the concept of a crystal, says Wayne Saslow, a theorist at Texas A&M University in College Station. Ordinarily, atoms in a crystal stack into rows like billiard balls. But, thanks to quantum mechanics, light and lively helium atoms behave like extended waves that somehow overlap to create the corrugated crystal structure, Saslow says, and theorists must decipher how such a quantum solid works: "We don't know what a solid is. We only thought we knew."

In a Vacuum, No One Sees You Splatter

Nature may abhor a vacuum, but a vacuum abhors a mess. In the absence of air, a droplet of liquid can crash into a smooth surface without splattering, report physicists Lei Xu, Sidney Nagel, and colleagues at the University of Chicago, Illinois.

"I was very surprised to see [the splash] go away and this beautiful smooth spreading of the droplet emerge as the air pressure was reduced," says Mark Robbins, a theorist at Johns Hopkins University in Baltimore, Maryland. Robbins says he would have assumed that the splashing depended on the properties of the liquid alone.

The splash spits out a ring of smaller droplets, and Xu and Nagel were studying their sizes and speeds when they discovered that pumping away the surrounding air eliminated the splash altogether. Within a tall vacuum chamber, the researchers released droplets of alcohol from various heights onto a dry glass plate. They recorded the resulting splashes with a high-speed video camera as they varied the pressure in their apparatus,



Now you see it. A droplet in air (*left*) makes a splash; one in a vacuum spreads smoothly.

CREDITS (TOP TO BOTTOM): JOHN PASSANEAU/PENNSYLVANIA STATE UNIVERSITY; LEI XU/UNIVERSITY OF CHICAGO

sucking it down as low as 1% of atmospheric pressure. The droplets struck the surface with speeds ranging from 2 to 7 meters per second, and for a given speed, the researchers found they could suppress the splash by lowering the pressure below a specific threshold.

The researchers explain the results with a simple theory. As a drop strikes the surface, liquid spreads sideways at supersonic speed, creating a shock wave. The shock wave pushes back on the liquid, and if that force is greater than the internal forces holding the liquid film together, the shock wave lifts it off the surface and creates a splash. Reducing the pressure reduces the force the shock wave exerts.

Ironically, the theory predicts that a thicker liquid should splash more easily than a thinner one. The researchers tested this prediction by dropping three types of alcohol with different viscosities. As predicted, the more viscous the alcohol, the lower the pressure needed to prevent splashing, the researchers reported. They also confirmed that a weighty gas such as krypton produced splashes at lower pressure than a lighter gas such as helium did.

"It's just the sort of thing all physicists should do," says Walter Goldburg, an experimenter at the University of Pittsburgh, Pennsylvania. "They spotted a nonintuitive phenomenon and pursued it" to a complete understanding. Xu and Nagel speculate that the odd phenomenon could make a splash with technologists, as it might be used to control splatter in industrial processes such as spray coating and inkjet printing.

Snapshots From the Meeting

Broadcasting with nantennae. Individual polymer molecules can stand on end and radiate light in exactly the same pattern that radio towers pump out radio waves, reports Michael Barnes, a chemist at the University of Massachusetts, Amherst. When excited with laser light, each fluorescent molecule pumps out photons in a "dipole" distribution, just as an antenna broadcasts radio waves. Within so-called nanophotonic devices, the "nantennae" might be lined up in "phased arrays" that would allow them to radiate in concert and direct their light in specific directions, Barnes says.

Energy: The stuff of confusion. Nearly half of college students come away from introductory physics classes thinking energy is a material substance, reports Michael Loverude, a physicist at California State University, Fullerton. Studying quiz and exam answers from classes for nonmajors, Loverude found that many students believe, for example, that a battery grows noticeably lighter as it runs down, implying that energy is a weighty thing. Andrew Boudreaux, a physicist at Western Washington University in Bellingham, notes that even physicists talk as if energy were a substance. "If you tried not to," he says, "you'd be talking in a very abstruse way."

Play your favorite proteins. Soon, identifying proteins may be as simple as slapping a compact disk into a specialized CD player, reports David Nolte and colleagues at Purdue University in West Lafayette, Indiana. The researchers have developed BioCD, a compact disk covered with gold spokes plated with various molecular targets. When molecules bind to spokes adorned with a particular target, they alter laser light reflecting off the disk. The technology should be more quantitative and faster than fluorescence bioassays, Nolte says, and could potentially assay 10,000 proteins at once. **–A.C.**

Recipe for Flies' Eyes: Crystallize

The striking hexagonal pattern in a fly's compound eye forms in the same way that a crystal grows, say physicists who have modeled the chemical interactions driving the process. The layered pattern of atoms in a crystal emerges as additional atoms nestle into the dimples between those in the previous layer. In the same way, the pattern in the larval fruit fly's eye emerges as each new eyelet, or ommatidium, fits into a gap in the previous row of elements, reports David Lubensky of the Free University of Amsterdam in the Netherlands. The step-by-step process produces the delicate pattern without a detailed blueprint.

The results support the notion that short-range signaling determines structure, says Albrecht Ott, a physicist at the University of Bayreuth in Germany. "Short-range communication is just so easy," Ott says. But researchers agree that the model must be tested experimentally.

The mechanism may be another tool with which nature sculpts living forms. The basic structure of the fruit fly's body is determined by gradients in the concentrations of proteins in the embryo. The gradients tell each cell where it is and what genes it should express. But that mapping scheme does not account for the eye pattern, says Boris Shraiman of the University of California, Santa Barbara. With hundreds of ommatidia, the eye has too many parts for such a mechanism, he says, and the genes involved do not produce static protein gradients.

Row by row. A fly's eye grows the way a crystal does, according to a chemical model.

So Lubensky and Shraiman tried to create the pattern another way. In 1952, British mathematician Alan Turing proposed that patterns form within a developing organism when a pair of chemicals, an "activator" and an "inhibitor," diffuse into each other and engage in a tango that leads to regions alternately rich in each chemical. The different regions then trigger different types of growth. The Turing mechanism appears to explain zebra stripes and other pigment patterns, but Lubensky and Shraiman couldn't make it produce the pattern in the fly's eye.

Instead, they found that each budding ommatidium simply nestles itself into the space provided by its neighbors. The transcription factor *atonal* controls the process. A wave of *atonal* expression moves across the undifferentiated eye disk, leaving behind spatially separated individual cells expressing *atonal*, which seed the ommatidia. Interactions between *atonal* and diffusing proteins such as *scabrous* that inhibit it determine which cells continue to express *atonal*, Lubensky and Shraiman report. Using experimentally determined relations between *atonal* and such inhibiting factors, they modeled the process and found that, instead of producing a specific pattern with a definite spacing—as the Turing mechanism would do—the interactions merely ensured that each row of ommatidia patterned itself after the previous one.

Now researchers are looking for ways to test Lubensky and Shraiman's model. For example, the model predicts that disrupting signaling between emerging rows of ommatidia even temporarily should cause the pattern to change dramatically across the rest of the eye—something that would not happen if the Turing mechanism were at work, says Herbert Levine, a theorist at the University of California, San Diego. Nicholas Baker, a molecular biologist at the Albert Einstein College of Medicine in New York City, has collected preliminary data that support the conjecture, Lubensky said at the meeting.

–ADRIAN CHO

CREDIT: STONE/GETTY IMAGES

Ski Mars, While There's Still Time

LEAGUE CITY, TEXAS—Scientists with interests in the rocky and icy bodies of the solar system met here from 14 to 18 March to focus on the ever-popular Mars and new data returned from the Saturn system by Cassini-Huygens.

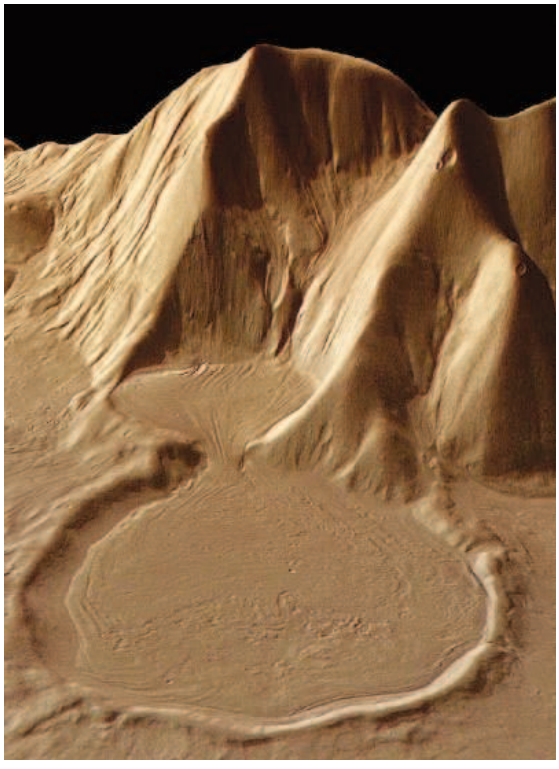
The Mars rovers picked up plenty of signs of long-ago water, but except for the ice of polar regions, reports of actual martian water—water ice under even tropical conditions—have been scarce. Now, however, with torrents of new imaging as well as new search strategies, researchers are seeing striking evidence of full-blown glaciers, some of them near the martian equator. Although they're not likely to be flowing today, many of these now-buried glaciers probably still contain ice laid down during the last great ice age of Mars more than 5 million years ago.

The key to the renaissance in martian glaciology is "new data seen with new eyes," says planetary geologist James Head of Brown University in Providence, Rhode Island. The new data have come from instruments aboard the Mars Global Surveyor and the camera on Mars Express.

The new eyes came from realizing that martian glaciers wouldn't look like most glaciers on Earth. Unlike most terrestrial glaciers, which slip along a wet layer at their base and bulldoze everything in their path, the far colder martian glaciers would ooze ever so slowly over the landscape rather than through it. Head and glaciologist David Marchant of Boston University in Massachusetts have found glaciers like that in Antarctica's deeply frigid Dry Valleys. "When you're down there, you feel you're on Mars every day," says Head.

The features researchers are seeing on Mars are "just dead ringers for what we see in the Antarctic Dry Valleys," says Head. He and Mars Express colleagues reported at the meeting that they recognize glacial flows off high massifs east of the Hellas Basin at 40° south and at the base of lofty Olympus Mons at 18° north. Head and his Brown University colleagues showed a large depression at 39° north whose ridged floor appears to be converging on a gap in its southern rim and flowing out. And Ernst Hauber of the German Aerospace Center in Berlin and Mars Express camera team members showed that glacial deposits fill a volcanic crater on the side of Hecates Tholus at 30° north. The list of glacial presentations goes on.

"I'm pretty impressed," says Mars geologist Michael Carr, emeritus researcher at the U.S. Geological Survey in Menlo Park, California. Ice does seem to have flowed across parts of Mars, he says. Glaciers aren't forming there today, most researchers agree, because the current climate locks up too much water at the poles. But to judge by the scarcity of impact craters, these glaciers were flowing in the geologically recent past—say, millions to tens of millions of years ago. Back then, calculations show, Mars was tilted much farther on its side, which would have allowed the sun to warm the poles and drive some of their water toward the equator.



The hourglass. A glacier appears to have descended from this 4-kilometer-high mountain (height exaggerated by 30x).

Some of that ice is probably still where it stopped flowing when the climate changed, researchers say. They don't usually see the pits and crevasses that would mark the loss of underlying ice back to the atmosphere by sublimation. Apparently, surface layers of rock and dirt have insulated the ice and preserved it for millions of years, as similar debris has done on Dry Valley glaciers. When Mars Express deploys the antennas of its ground-penetrating radar in early May, researchers may get to the bottom of the glaciers of Mars.

Rovers, Dust, and a Not-So-Wet Mars

The Red Planet could just as easily be called the Dust Planet. Dust is pretty much everywhere on Mars. It gives the planet its color (actually more of a yellowish brown than red), and it coats almost everything it doesn't bury. Most scientists had assumed that Mars's dust was the end product of some sort of planetary rusting, but researchers examining dust captured by magnets on the Mars rovers have found that dust's magnetic component is mainly pristine magnetite, the shiny-black mineral of lodestones. Despite all the talk about how wet Mars has been, even tiny bits of its rock have escaped eons of weathering unscathed. That supports an emerging picture of a Mars wet mainly in its early days—and then only wet intermittently.

The dust discovery comes from the Magnetic Properties Experiments (MPE), a set of variously shaped magnets of differing strengths mounted on the Opportunity and Spirit rovers. Morten Bo Madsen of the University of Copenhagen, Denmark, and his MPE teammates reported at the meeting that the way atmospheric dust has accumulated on the magnets means that nearly all of the dust particles must contain at least a trace of a strongly magnetic mineral, which two of the rovers' arm-mounted analytical instruments identified as magnetite.

Magnetite had not been the leading contender for martian magnetic dust. Yellowish brown maghemite, another iron oxide, was presumed to be the magnetic component after the Viking and Pathfinder missions. It had the right color, fit the reported oxidizing condition of martian soil, and could have formed in hot springs or by water weathering of exposed rock. Magnetite, on the other hand, must be unchanged from the day it crystallizes from molten rock.

Finding magnetite apparently eroded unaltered from the rock is consistent with other recent findings on Mars. Geologist Matthew Golombek of the Jet Propulsion Laboratory in Pasadena, California, and rover teammates reported at the meeting that about 10 centimeters of the 3-billion-year-old floor of Gusev Crater where Spirit landed has eroded away,

CREDIT: DLR/HRSC/ESA

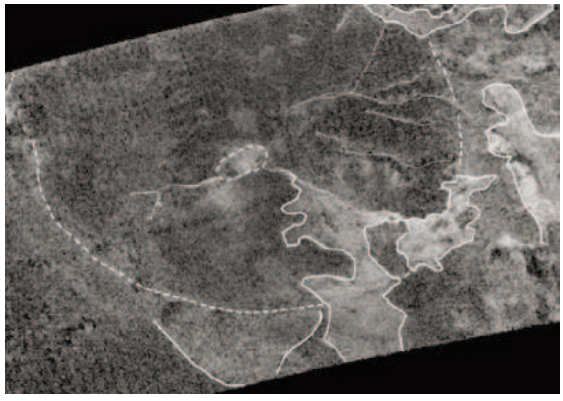
presumably by the wind. Water-dominated erosion on Earth, even when it's slow, is 50,000 times faster than that, Golombek noted. That implies that on Mars, "a dry and desiccating environment similar to today's" has been active for about 3.7 billion years.

Several presentations at the meeting considered how the expanse of salt deposits Opportunity discovered could have come about if only scant amounts of acidic water were available to erode rock on early Mars. The much discussed "shallow seas" of early Mars now more often appear as intermittent puddles among normally dry dunes. The Dust Planet may have been dusty for a long, long time.

Icy Volcanism Has Rejuvenated Titan

With a surface temperature of 179°C below zero and having had more than 4 billion years for its inner fires to damp, Saturn's icy moon Titan might seem an unlikely place to find lively geology. But the Cassini spacecraft returned promising images when it passed by the moon late last year and again in February. After further analysis, Cassini team members at the meeting could confidently point to "ice lava" flows, a huge volcanic ice dome, and possible ice volcanic calderas. Titan does seem able to resurface itself volcanically, which would help explain its surprisingly youthful appearance. Now planetary physicists just have to figure out how Titan still does it after all these years.

Two of Cassini's instruments pierced Titan's obscuring haze to return detailed images of cryovolcanic features. The Cassini radar produced images by bouncing microwaves off long strips of terrain. Rosaly Lopes of the Jet Propulsion Laboratory in Pasadena, California, and her radar teammates found a 180-kilometer-wide, flat-topped dome, Ganesha Macula, which bears a striking resemblance to the rocky volcanic domes of fiery-hot Venus. Ganesha Macula has a calderalike central depression, a radar-bright, flowlike feature spreading from center to edge, and four apparent channels meandering outward. One of them runs for more than 90 kilometers and may debouche near the dome edge as an icy lobate flow. And elsewhere in the radar strip, apparent flows extend as far as 200 kilometers, in two cases emanating from presumed volcanic craters.



Icy eruptions. Radar revealed 180-kilometer-wide Ganesha Macula, a broad dome strewn with "lava" flows.

At haze-piercing near-infrared wavelengths, the Visual and Infrared Mapping Spectrometer (VIMS) returned relatively high-resolution images of a few small parts of Titan, one of which contains a so-far-unique spiraling feature dubbed "the Snail." Thirty kilometers wide, it sports a central dot that's dark to radar and therefore presumably smooth. Sébastien Rodriguez of the University of Nantes, France, and VIMS teammates take the Snail to be a possible volcanic dome with a central caldera.

So cryovolcanic eruptions have probably resurfaced at least some parts of Titan. That's not completely unexpected. Unlike Saturn's Enceladus (*Science*, 4 March, p. 1387), Titan has plenty of rock deep within it that is still generating substantial heat from radioactive decay. Its ice could melt tens or hundreds of kilometers down, says planetary physicist David Stevenson of the California Institute of Technology in Pasadena. And ammonia thought to be within the moon could lower the melting point of ice, allowing water-ammonia lavas to flow on the surface, much as molten-rock lavas flow on Earth.

But Stevenson does wonder how Titan could still be flooding its surface with cryolavas this late in its life. No impact craters have been seen on any cryovolcanic features, implying that they are relatively young. Yet, notes Stevenson, billions of years of volcanic activity would have extracted the interior's reservoir of ammonia. Without that antifreeze, cryolavas could not flow. If cryovolcanism has indeed been geologically recent, Titan must be somehow recycling its ammonia back into the interior, he says.

–RICHARD A. KERR

Snapshots From the Meeting

Genesis reborn. After the Genesis spacecraft smashed into the Utah desert floor last September, team members hoped to recover at least some of the solar wind particles the craft had collected in deep space. At the meeting, the mission's first solid results bore out that optimism. "We see solar wind" in Genesis collectors, said team member Daniel Reisenfeld of the University of Montana, Missoula, "and we can measure it quantitatively and accurately." It won't be easy, though. The 10,000 separate pieces of shattered collection surface are covered with billions of micrometer-size bits of desert dirt. There's even a "brown stain" of goo vaporized from the spacecraft and deposited on some of the collectors while in space. Still, said principal investigator Donald Burnett of the California Institute of Technology in Pasadena, "we're not giving up on anything we wanted to do before the crash."

A younger Mars? Meteoriticist Ralph Harvey wants to redefine planetary old age. At the meeting, Harvey, who works at Case Western Reserve University in Cleveland, Ohio, and spectroscopist Victoria Hamilton of the University of Hawaii, Honolulu, concluded from spectroscopic data that the sprawling Syrtis Major volcanic center on Mars has the same distinctive iron- and magnesium-rich composition as the Nakhla/Chassigny group of eight known martian meteorites recovered on Earth. Syrtis Major might be the elusive source of these martian rocks, they said. They even point to a crater from an impact that could have launched Syrtis Major rock toward Earth. But the martian meteorites congealed from lavas 1.3 billion years ago, whereas counting the impact craters accumulated on Syrtis Major gives an age of 3.3 billion years for its rock. If the martian meteorites in fact came from Syrtis Major, Harvey and Hamilton note, crater counters have overestimated all martian surface ages by a factor of 2 to 3. A younger Mars would mean, among other things, that the planet was wet through most of its history rather than just in its earliest days.

Meteorite launcher? An impact on Syrtis Major of Mars may have blasted rock to Earth.

Spirit rover rejuvenated. Life was hard for the Spirit Mars rover early last month. So much dust had settled on its solar panels that the intrepid explorer was down to less than half its original power supply—not far from the "death zone," said rover team leader Steven Squyres of Cornell University in Ithaca, New York. Then came the miracle. From one Mars day to the next, Spirit's power bounced back to 90%. The rover's panoramic camera showed panels so clean they looked like the rover was "just off the showroom floor," quipped one team member. Opportunity, Spirit's twin on the other side of the planet, had enjoyed similarly mysterious solar panel cleanings. Spirit, Squyres reported, may have been swept clean by blustery winds while perched on a high ridge in the Columbia Hills. –R.A.K.

CREDITS (TOP TO BOTTOM): LOPES *ET AL.*/JPL/NASA; THEMIS/U. OF ARIZONA/NASA

2005 Tyler Prize

Charles David Keeling

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Tyler Prize Executive Committee announces the awarding of the 2005 Tyler Prize for Environmental Achievement on its thirty-second anniversary to Dr. Charles David Keeling, Professor, Scripps Institution of Oceanography, University of California, San Diego and Dr. Lonnie G. Thompson, University Professor, Byrd Polar Research Center of Ohio State University. Drs. Keeling and Thompson are recognized for their pioneering research, which has laid the foundation for and provided the clearest evidence of the growing impact of global climate change.

Charles David Keeling is recognized for his rigorous time series measurements of atmospheric carbon dioxide and their interpretation. These carefully made observations conducted over four and a half decades, and continued today, have revealed world wide increases in carbon dioxide with striking spatial and temporal patterns of variability that show relationships between the carbon cycle and climate, and reveal unanticipated links between these components and the earth system. From his remarkable lifetime of scientific investigations, we know that humans are altering the global physical environment. Web:http://earthguide.ucsd.edu/globalchange/keeling_curve/01.html

Lonnie G. Thompson is recognized for his pioneering work in the collection and analysis of valuable climatic information contained in tropical glacier ice cores from all over the world. These tropical ice cores have provided understanding of paleoclimatic conditions against which current climate changes can be compared. The high altitude collection of these evidences of past climatic conditions is a heroic feat of mountaineering that requires courage, daring and physical endurance comparable to the legendary explorers of yore. Web:http://www.geology.ohio-state.edu/modules.php?op=modload&name=Faculty&id=thompson.3@osu.edu&file=faculty.profile

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Random Samples

Edited by Jocelyn Kaiser

Ancient disinfection.

Lifesaving Pitchers

People in rural India have long believed that storing water in brass pitchers can ward off illness. A new study backs that idea, finding that traditional brass pitchers release tiny amounts of copper that kill harmful bacteria.

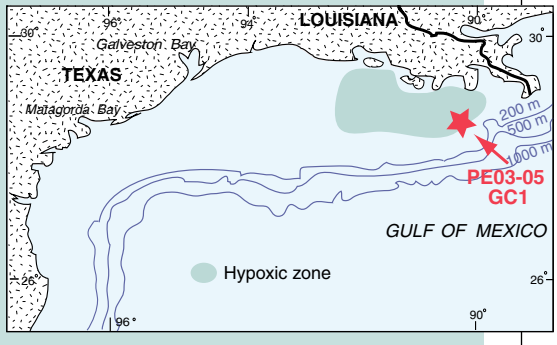
Rob Reed of Northumbria University in Newcastle, U.K., verified the pitchers' powers with collaborators at Panjab University in Chandigarh, India. The microbiologists filled brass pitchers with sterile water inoculated with *Escherichia coli* and with contaminated river-water samples collected in India. Fecal bacteria counts in all samples dropped from as high as 1,000,000 bacteria per milliliter to zero after 2 days. Bacteria levels stayed high in water stored in earthenware or plastic pitchers. The brass pitchers contained traces of dissolved copper, enough to kill bacteria, Reed reported this week at the Society for General Microbiology annual meeting in Edinburgh, U.K. The study, he notes, "supports an ancient, anecdotal kind of belief."

Gulf's Dead Zone Worse in Recent Decades

A seasonal dead zone in the northern Gulf of Mexico developed occasionally in the 1800s, according to a new study. But its data suggest that the zone has become more intense in the last few decades as farmers cranked up fertilizer use.

Coastal bottom waters off Louisiana now become depleted of oxygen almost every summer when nutrient-rich Mississippi River water causes populations of tiny marine plants called phytoplankton to explode. When they die, their decomposition sucks oxygen from the bottom waters. Fish and other animals then flee the area.

Most scientists believe that chemical fertilizer is a major cause of the seasonal dead zone, but the fertilizer industry and a few scientists are skeptical (*Science*, 9 February 2001, p. 968). To probe past conditions, a team led by micropaleontologist Lisa Osterman of the U.S. Geological Survey in Reston, Virginia, took sediment cores from the consistently hypoxic zone. They dated cross sections and counted three species of tiny animals called foraminifers that tolerate low-oxygen waters.



Coring spot.

As far back as 1823, the hardy foraminifers thrived during Mississippi River flood years, suggesting that nutrients in floodwaters can trigger natural hypoxia. But the foraminifers were much more abundant after 1960, when Mississippi River Basin farmers began laying on commercial fertilizer. That has apparently driven low-oxygen episodes "very far off scale," says Osterman, whose study appears in the April issue of *Geology*.

Although intrigued by the study, marine biologist Robert Diaz of the Virginia Institute of Marine Sciences in Gloucester Point cautions that the sediment-dating technique used can be off by a few years.

Fastest DNA Computer

A biomolecular computer that uses little more than DNA and enzymes could perform a billion operations simultaneously, say scientists led by Ehud Keinan of the Technion-Israel Institute of Technology in Haifa.

Three years ago, a joint team from Technion and the Weizmann Institute of Science in Rehovot, Israel, published a paper in *Nature* on a DNA-based computer. But the machine was limited to only 765 simultaneous programs and, unlike this new system, it required human supervision.

The new biomolecular computer is described in the March 2005 issue of the *Journal of the American Chemical Society*. Computations are carried out by processing the input (double-stranded DNA molecules) with the help of enzymes that chop and reassemble the DNA in a series of steps. The output is in the form of a slightly altered DNA molecule.

The system's complexity "is certainly novel and [has] never been achieved before," says Natasha Jonoska, a mathematician at the University of South Florida, Tampa.

Fish Farm Hazards

Parasitic sea lice that jump from fish farms to wild salmon may be a much greater problem than suspected, according to a new report likely to inflame an ongoing battle over aquaculture risks.

The idea that fish farms act as reservoirs of infection for passing wild fish is controversial in the United States and Canada. To test the theory, researchers at the University of Victoria (UVic), Canada, looked for lice on more than 5000 juvenile wild pink and chum salmon along a migration route close to a fish farm in British Columbia. The scientists plugged their lice counts, along with environmental parameters, into a mathematical model.

Killer sea lice.

The results, in the 29 March issue of the *Proceedings of the Royal Society*, indicate that the juveniles started out generally lice-free but became heavily infected after passing the farm. And the lice held on for 30 kilometers, making the "migrating school a moving cloud of infection" that can be passed to other species such as stickleback and herring, says John Volpe, a marine ecologist at UVic.

"This is an excellent paper," says Ransom Myers, a fisheries biologist at Dalhousie University in Halifax, Nova Scotia. But Scott McKinley, a physiologist at the University of British Columbia in Vancouver, calls the study "flawed" because it lacks "lice data from the farm itself."

CREDITS (TOP TO BOTTOM): ROB REED; LISA OSTERMAN/USGS; ALAN PIKE

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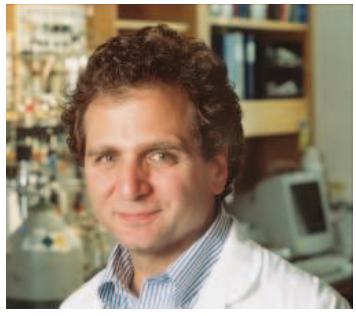
Edited by Yudhijit Bhattacharjee

Taking Stock at NIH



The new conflict-of-interest rules at the National Institutes of Health (NIH) are driving one institute director to leave and delaying the arrival of another. James Battey (left), director of the National Institute on Deafness and Other Communication Disorders, has told NIH officials that he plans to quit before the provisions concerning investments take effect this fall. And lung disease researcher David Schwartz of Duke University in Durham, North Carolina, who was to take the helm next week at the National Institute of Environmental Health Sciences (NIEHS), is having second thoughts about the position.

Battey says he is unable to comply with the ban on senior employees owning biomedical stocks because he manages a family trust fund. "I can't abandon that responsibility," he says. The same ban is deterring Schwartz, who sent a letter to NIH Director Elias Zerhouni expressing his concerns about its possible effect on recruitment and retention of scientists. In an e-mail to *Science*, Schwartz said he still plans to come to NIEHS and is "confident that my concerns can be addressed."

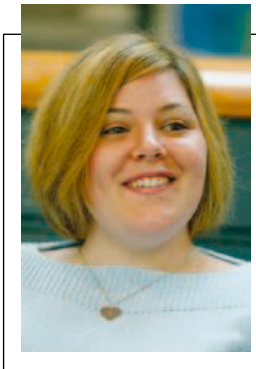
Battey was promptly removed from his post as chair of the NIH Stem Cell Task Force last month after he told NIH officials about his plans to leave. His next job could take him back to his native California: He says he's "one of many candidates" for a top position at the California Institute for Regenerative Medicine, which will distribute the state's $3 billion Proposition 71 funding for stem cells and cloning. Allen Spiegel, director of the National Institute of Diabetes and Digestive and Kidney Diseases, and Story Landis, director of the National Institute of Neurological Disorders and Stroke, have been named co-chairs of the Stem Cell Task Force.

IN THE NEWS

Time to bloom. Early April is the time to enjoy Washington, D.C.'s premier spring event: the blossoming of thousands of cherry trees. It's also a time to doff your hat to horticulturist Robert DeFeo of the National Park Service, whose job is to predict when the flowers will be in full bloom.

For much of the winter, DeFeo checks the buds daily to gauge their readiness. One month before the Cherry Blossom Festival, he announces his best guess of when peak intensity will occur. Although the festival's dates have been chosen months in advance, DeFeo's predictions help countless tourists and local residents plan their sightseeing. This year's prediction, 4 to 9 April, is his 15th, 13 of which have been right on the mark despite the erratic March weather. "I am just amazed [at his accuracy]," says Margaret Pooler of the National Arboretum in Washington, D.C.

Got any tips for this page? E-mail people@aaas.org



THEY SAID IT

"They definitely hold a special place in my heart. I always considered them to be my second set of grandparents."

—Elizabeth Carr, the first test tube baby of the United States, on Georgeanna Jones and her husband Howard Jones, who pioneered in vitro fertilization in the country and established the program leading to Carr's birth. The 24-year-old Carr made the comment in an interview to *The Baltimore Sun* after Georgeanna Jones died in Norfolk, Virginia, on 26 March. She was 92.

CREDITS (TOP TO BOTTOM): DUKE UNIVERSITY MEDICAL CENTER; NIH; JULIA MALAKIE/AP; TERRY J. ADAMS/NATIONAL PARK SERVICE; THE ALAN MASON CHESNEY MEDICAL ARCHIVES OF THE JOHNS HOPKINS MEDICAL INSTITUTIONS

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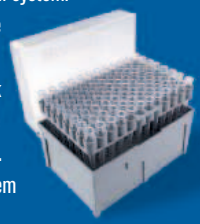

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Biodiversity in Sri Lanka and the Western Ghats

WE READ WITH INTEREST THE REPORT "LOCAL endemism within the Western Ghats–Sri Lanka biodiversity hotspot" by F. Bossuyt *et al.* (15 Oct. 2004, p. 479), which documents patterns of diversification in selected vertebrate and invertebrate lineages from Sri Lanka and the Western Ghats region of western India. Although these two areas have long been united as a single biogeographic unit (*1*), and more recently as a biodiversity "hotspot" (*2*), Bossuyt *et al.* highlight the distinctive faunal histories of the two regions and caution against treating them as a single unit for conservation purposes. We would like to add two comments, which support and extend their results.

First, the respective bird and mammal faunas of Sri Lanka and the Western Ghats are distinct in many ways: There are marked differences in the regions' restricted-range mammal assemblages [the Western Ghats support at least 15 endemic mammal species; Sri Lanka supports at least 13 endemic species, and because they share few restricted-range birds, they are treated as separate "Endemic Bird Areas" (*3*)]. This is significant because it is birds and mammals that tend to act as "flagship species" for conservation.

Second, trenchant faunal differentiation is evident within both areas, especially in different climatic zones within Sri Lanka (*4*, *5*), and the two regions can be subdivided into multiple "ecoregions" (*6*). There may sometimes be stronger faunal differentiation between wet, dry, and cloud forest zones within Sri Lanka than between that island's dry zone and the dry country of South India [e.g., (*4*)]. Lists of mammals restricted to Sri Lanka, the Western Ghats, or the hotspot as a whole are given in (*7*–*10*). Those apparently restricted to high-altitude cloud forest zones (marked with an asterisk) comprise all endemic genera, half of Sri Lankan endemics, one-third of Western Ghats endemics, and about one-third of mammal species endemic to the hotspot as a whole.

KRISTOFER M. HELGEN[1] AND COLIN P. GROVES[2]

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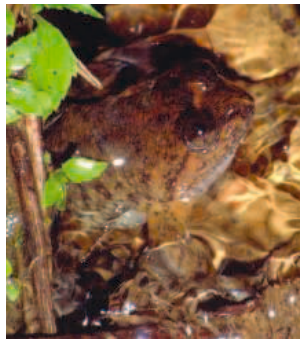
8. Shared exclusively: *Crocidura horsfieldii*, **Feroculus* cf. *feroculus*, **Suncus montanus, Ratufa macroura, Petinomys fuscocapillus, Funambulus layardi, Funambulus sublineatus, Herpestes fuscus, Herpestes viticollis.*
9. Western Ghats: *Paraechinus nudiventris, Suncus dayi, *Latidens salimalii, Macaca silenus, Trachypithecus johnii, Funambulus tristriatus, *Mus famulus, *Vandeleuria nilagirica, Rattus ranjiniae, *Rattus satarae, Platacanthomys lasiurus, Martes gwatkinsi, Paradoxurus jerdoni, Viverra civettina, *Nilgiritragus hylocrius.*
10. Endemic mammalian genera: Sri Lanka: **Solisorex, *Srilankamys*; Western Ghats: **Latidens, *Platacanthomys, *Nilgiritragus*; shared exclusively: **Feroculus.*

Response

HELGEN AND GROVES' POINT about conservation is well taken. Yet, the major significance of our study is that it reaches beyond the recognition of a high degree of species endemism. Indeed, we have demonstrated that several Sri Lankan taxa not only contain assemblages of endemics, but that these sometimes constitute old branches or distinct clades of the tree of life. Such higher-level endemism is also evident in ranid frogs (*Lankanectes*) (*1*), agamid lizards (*Ceratophora*) (*2*), and land snails (*3*). The island may therefore be considered a significant reservoir of ancient lineages and clade evolutionary history (*4*).



***Lankanectes*, an ancient frog lineage in Sri Lanka.**

CREDIT: F. BOSSUYT *ET AL.*

From a conservationist's point of view, this is significant because radiations of tens of species are found exclusively on Sri Lanka. Because some members of these evolutionary lineages can be readily viewed in gardens (e.g., *Philautus* treefrogs) or in roadside torrents (e.g., parathelphusid freshwater crabs), they are ideal catalysts for stimulating environmental awareness.

With few possible exceptions (mice and shrews), mammals and birds do not show clade-level endemism on Sri Lanka. Therefore, conservation managers could treat the clades of animals and plants as the island's major natural treasure, instead of selecting a single mammal or bird as a flagship species. This strategy will reinforce the fact that not only selected sites, but the island's habitats as a whole deserve protection.

It is in that perspective noteworthy that Sri Lanka's diversity is largely restricted to the formerly rain-forested southwestern "wet zone," where only ~750 km^2 of (highly fragmented) natural forest now survives. Human population density in Sri Lanka is one of the highest of all Global Biodiversity Hotspots (*5*). The threats to the unique biodiversity we uncovered, and the challenges to its conservation, are therefore formidable and demand urgent international scientific attention.

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What Kind of Science Is Biology?

H. O. SIBUM'S THOUGHT-PROVOKING ESSAY "What kind of science is experimental physics?" (1 Oct. 2004, p. 60) hinges on a tension voiced by German theoretical physicist Felix Auerbach, who claimed that experimental physicists "invent," in contrast to biologists, who "discover." If inventing means generating new material arrangements, then certainly species invent, for instance, when bacteria evolve drug resistances. We suggest, then, that biologists discover things that species invent.

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Response

I WILL TRY TO PROVIDE AN ANSWER TO THE question posed by Myers and Madjid in the title of their Letter by expanding on the arguments provided by Auerbach. The distinction made by Auerbach between "invention" and "discovery" points to an interesting methodological problem that has accompanied experimental physics since its beginnings in the 17th century. In the 18th century, you could use discovery and invention synonymously—e.g., you could say, "I invented longitude." Only in the 19th century was a clear divide between discovery and invention made: The former designated the scientists' endeavor and the latter the engineers' approach. Around 1900, microphysics in particular sparked a renaissance of self-reflexivity among physical scientists about their methods because new scientific objects such as electrons, x-rays, and so forth became visible only through human-built devices. According to Auerbach this "technical science" produced "physical phenomena" rather than "natural phenomena." In the English language, this distinction does not work, but in the German context, Auerbach's linguistic discrimination between "physikalische Phänomene," understood as effects produced in the physics laboratories, and "Naturphänomene," understood as effects observed in nature, pointedly marks his epistemological stance: Methodologically speaking, experimental physicists had become inventors in the engineering sense. Therefore, for Auerbach, experimental physicists were no longer mere "observers of nature" but inventors engaged in the creation of "artificial experiments," whereas botanists or geologists were observatory scientists and therefore discoverers.

Myers and Madjid's suggestion to treat species as actors who invent makes a lot of sense. But what role do the biologists in their laboratories play? Are they, in the Auerbach sense, like 19th-century botanists, mere discoverers? Are they, as Myers and Madjid suggest, passive observers of how species invent? Or is it not the case that biologists through their technical science equally set the stage for these species to act? Therefore, it would be rather enlightening to reflect on experimental biologists as inventors too.

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Conduct and Reporting of Clinical Research

In his Editorials "Clinical trials and public trust" (3 Dec. 2004, p. 1649) and "The old file-drawer problem" (23 July 2004, p. 451), Donald Kennedy discusses the problem of poor-quality trials funded by pharmaceutical companies in the wake of the Vioxx controversy and the need for improved registering and monitoring of clinical experiments. This problem goes beyond the pharmaceutical

industry. A recent empirical study showed that 62% of randomized controlled trials deviated from their research protocols in reporting primary outcomes (*1*). For both harm and efficacy data, outcomes were more likely to be reported if they were statistically significant (*1*). This outcome-reporting bias also applied to trials funded by a reputable government research council (*2*). An effective registry is certainly needed, but publication of trial protocols is also strongly indicated (*1*–*3*). Journals may ask to see protocols, a requirement recently made by the *British Medical Journal* (*4*).

In dealing with the issue of drug safety, the CONSORT (Consolidated Standards of Reporting Trials) group has acknowledged the problem and recently released an extension to its 22-item checklist to include the reporting of harms as well as efficacy (*5*, *6*). There is a need to improve the reporting and conduct of clinical research, even those funded by government research councils, reputable charities, and universities, in addition to improved monitoring procedures by regulatory agencies such as the U.S. Food and Drug Administration.

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Comparative Studies of Drug Efficacy

I HEARTILY AGREE WITH THE SUGGESTION OF commissioned studies for the safety of pharmaceuticals, given in the News Focus article "Gaps in the safety net" (J. Couzin, 14 Jan., p. 196). Some commissioned studies that compare new drugs' efficacy with that of previously available alternatives would also be in the public interest. We physicians need help in selecting the most cost-effective agent among several very similar competing products.

A reform that the pharmaceutical industry might find agreeable would be to stop the clock on patent expiration from the moment of FDA approval of a new drug until the accumulation of sufficient safety data about it, or until the manufacturer chooses to begin promoting the product commercially (whichever occurs first). This type of extension of patent protection would

tend to get public and corporate benefits back in step. After all, patents originated to promote innovation for the public's benefit.

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Does the Dose Make the Poison?

RANDOMIZED CLINICAL TRIALS (RCTS) ARE performed to provide unbiased assessment of the risks versus benefits of therapeutic and chemopreventive medications. Recently, two highly publicized RCTs noted increases in the risk of adverse cardiovascular outcomes with intake of well-known selective COX-2–inhibiting agents (rofecoxib and celecoxib) ("Gaps in the safety net," J. Couzin, News Focus, 14 Jan., p. 196). These studies were designed to assess chemopreventive effects of fixed doses of drugs against the recurrence of colonic polyps. Dosages of both rofecoxib (Vioxx) and celecoxib (Celebrex) administered in these RCTs were above the standard recommended doses (8 and 16 times, respectively, the typical dose when used in the treatment of arthritis). The nature of the double-blinded experimental design for these RCTs did not allow for adjustment of dose according to body size as recommended by the drug manufacturers. Because the therapeutic window of smaller individuals is usually reduced, their dose should be lowered and safety tolerance checked by measuring individual blood levels. Experimental designs of clinical trials should embellish rather than ignore two golden rules of medicine: The dose makes the poison and first do no harm.

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TECHNICAL COMMENT ABSTRACTS

COMMENT ON "*Pierolapithecus catalaunicus*, a New Middle Miocene Great Ape from Spain"

David R. Begun and Carol V. Ward

Moyà-Solà *et al.* (Research Articles, 19 Nov. 2004, p. 1339) identified the new genus *Pierolapithecus* as a stem hominid (great ape and human clade) that engaged in little forelimb suspension. Our analysis indicates that *Pierolapithecus* is more probably a hominine (African ape and human clade). The trunk, wrist, and phalangeal morphology are consistent with well-developed suspensory behavior, but do not preclude palmigrady.

Full text at www.sciencemag.org/cgi/content/full/308/5719/203c

RESPONSE TO COMMENT ON "*Pierolapithecus catalaunicus*, a New Middle Miocene Great Ape from Spain"

S. Moyà-Solà, M. Köhler, D. M. Alba, I. Casanovas-Vilar, J. Galindo

A hominine status of *Pierolapithecus* is not supported by the characters used by Begun and Ward in their cladistic analysis. Long hands relative to body mass are considered to characterize specialized suspensory behaviors, while modern hominoid-like thorax and wrist morphology is associated with orthogrady and shared by all extant hominoids regardless of their species-specific locomotor adaptations.

Full text at www.sciencemag.org/cgi/content/full/308/5719/203d

CORRECTIONS AND CLARIFICATIONS

Special Issue on Einstein's Legacy: News: "Special relativity reconsidered" by A. Cho (11 Feb., p. 866). The affiliation of V. Alan Kostelecký was misstated. He is at Indiana University.

In Search of Time

Naomi Gerstel

The Time Divide
Work, Family, and Gender Inequality
by Jerry A. Jacobs and Kathleen Gerson

Harvard University Press, New York, 2004. 259 pp. $45, £29.95, €41.50. ISBN 0-674-01153-8.

Working in a 24/7 Economy: Challenges for American Families
by Harriet B. Presser

Russell Sage Foundation, New York, 2003. 281 pp. $39.95. ISBN 0-87154-670-1.

At the turn of the 20th century, Americans boasted about their leisure. Entering the 21st, they brag about how busy they are. But not everyone is happy about being so busy. Debates about compensation for overtime, who qualifies for overtime, the availability of family job leave, and the safety of long work hours and night shifts have been the subject of some of the sharpest political conflicts of recent times—vigorously contested in Congress, in presidential elections, and at collective bargaining tables. Until very recently, however, these arguments have been based largely on preconceived ideas, with little grounding in systematic research.

Two recent books—Jerry Jacobs and Kathleen Gerson's *The Time Divide*, about job hours, and Harriet Presser's *Working in a 24/7 Economy*, about work schedules—go a long way toward informing these debates. Taken together, they show that social class is a key source of variation in the temporal organization of work. Hours and schedules squeeze at opposite ends of the class structure: affluent employees tend to work long hours but often on "standard schedules"; those farther down the occupational hierarchy work fewer hours but tend to do so on nonstandard schedules.

Using various national data sets, the books rebut two widely cited arguments. One, put forward most prominently by economist Juliet Schor, is that Americans work longer hours than those in other countries and that work hours in the United States have increased dramatically over the past few decades (*1*). The other, suggested by Arlie Hochschild, claims that employees work such long hours on odd schedules because they look to their jobs as an escape from the stresses of family life (*2*). Companies, Hochschild argues, make it possible to work less time, but workers actually prefer to work long hours.

A substantial fraction of U.S. workers do put in long hours, and in the last few decades, they have increased the weeks per year that they work. But, as Jacobs and Gerson (sociologists at the University of Pennsylvania and New York University, respectively) show convincingly, the average length of the workweek has remained largely unchanged since 1970. Instead, they find that it is "variation around the average [that] has increased." Looking at individual workers, they show that the time squeeze created by long hours at work is concentrated among professionals and managers to whom the media and public so often attend. A much smaller proportion of blue-collar workers spend long hours on the job. Jacobs and Gerson write of this growing bifurcation: "An occupational divide is developing between jobs that demand excessive time commitment and jobs that may not offer sufficient time at work to meet workers' needs or preferences."

If there has not been an increase in the average hours of workers, is there no real change—just louder complaints, more bragging, and more attention to those complaints? No, there is plenty of change. To understand it, Jacobs and Gerson point toward families rather than individual workers. Most of the growth in annual hours on the job reflects the growing employment of women, more and more of whom are committed to full-time year-round jobs. This means an upsurge in time squeezes for families: with more women in the labor force, there are more families with two earners, producing a higher proportion of families with many hours of combined work time. We have also witnessed increases in divorces, in babies born outside of marriage, and, as a result, in single mothers. Most of these mothers feel a constant tug between the need to support their children and the need to spend time with them. Two-earner couples and single parents have always, according to Jacobs and Gerson, faced a time bind. It is the increasing prevalence of such families, rather than an overall upsurge in the average individual job hours, that has caused the stir about work time.

A prominent image of American life still has parents or partners coming home in the evenings and on weekends—perhaps exhausted and anxious, but nonetheless spending these hours at (or at least near) their homes. From Presser's *Working in a 24/7 Economy*, however, we learn that only a small and shrinking majority (54%) of U.S. citizens work a fixed daytime Monday-to-Friday schedule; a majority of two–wage earner couples include at least one spouse who works weekends. Relying on large national surveys, Presser (a sociologist at the University of Maryland) shows that a growing number of people work nonstandard schedules that include evening, night, or weekend hours. The term "nonstandard schedules" is fast becoming a misnomer, with so-called "standard" schedules increasingly a style of life reserved for a shrinking pool of privileged U.S. families. Like work hours, work schedules are organized by class; however, long hours are most common at the high end of the occupational structure, whereas nonstandard work schedules are disproportionately concentrated in jobs low in the occupational hierarchy.

Are time-squeezed employees working such long hours, at odd schedules, to escape their families, as Hochschild proposed? Both these books suggest not. Jacobs and Gerson find that those who work long hours—professionals and managers—are particularly likely to say they would prefer to work fewer hours. Both women and men want to spend fewer hours on the job. Parents, whether they have young children or teenagers, want more time at home. But they are captured by "greedy institutions" where those in power extract people's time and energy by instilling demands for loyalty and by undercutting people's loyalty to other institutions (most notably, families).

What of odd schedules? Some suggest that nonstandard schedules are a result of

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the desires on the part of mothers and fathers to do tag-team parenting, to avoid having to hire strangers to care for their kids, or to increase income by accepting shift work. This is not the case, according to Presser. Just as most people do not want to work long hours, most people do not prefer nonstandard work schedules. Instead, those with such schedules have little choice: the only jobs they can find not only pay lower wages but also require that they work evenings, nights, or weekends. But their marriages suffer. Presser finds that whether it is the wife or husband in dual-earner couples who works a non-day schedule, both partners are likely to say their marriages are less happy, and they are more likely to get a divorce (at least when they have children) than those with more conventional day schedules.

What about the kids? Nonstandard work schedules mean families have dinner together less frequently, family members engage in private talks less often, and parents give less help on homework. According to Presser, these couples enjoy one advantage: the husbands do more housework and provide more childcare. To be sure, compared to those who work days, parents who work evenings, nights, or rotating schedules are also more likely to share family breakfasts and to be home when children leave for or return from school. But after odd schedules and long nights, are such parents awake? Are they exhausted? Unfortunately, that kind of information is not available in the national data sets available to Presser. But among the virtues of Presser's book are her own acknowledgment and assessment of the limits of available data, her calls for future research, and her specification of what such research should look like.

The Time Divide and *Working in a 24/7 Economy* are indispensable references on working time. Each makes clear the need for revisions of U.S. social policy. Childcare is at the top of the list. Americans work much longer hours, on more "nonstandard" schedules, than Europeans, but the United States provides far less in the way of childcare—whether day care or afterschool care. The United States fares no better in comparisons of paid family leaves, overtime laws, or part-time work. Besides demonstrating the pressing need to rethink outdated U.S. policies about time, both books remind readers that the proposed policy fixes are not utopian visions but mandates that already exist in most of Western Europe.

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CREDIT: JOE SUTLIFF

SCIENCE AND RELIGION

The Blind Godmaker

Michael Shermer

In 1999, Frank J. Sulloway and I conducted a study on religious attitudes that included a question asking survey takers to explain in their own words why they believe in God. The most popular reason given was: "Good design, natural beauty, perfection, and complexity of the world or universe" (*1*). As pattern-seeking primates, we have a natural tendency to look for and find design in nature. Before 1859 the default explanation for that design was a top-down designer, God. This was most forcefully argued by the 18th-century English theologian William Paley: If one stumbled upon a watch on a heath, one would not assume it had always been there, as one might with a stone (*2*). A watch implies a watchmaker. Design implies a designer.

In 1859, Charles Darwin provided a scientific explanation of design from the bottom up: natural selection (*3*). Since then, arguably no one has done more to make the case for bottom-up design than the Oxford University evolutionary biologist Richard Dawkins in a series of books that includes the aptly titled *The Blind Watchmaker* (*4*), a direct challenge to Paley. But if design comes naturally from the bottom up and not supernaturally from the top down, what does that imply about the existence of God? Although most scientists avoid the question altogether or take a conciliatory stance along the lines of Stephen Jay Gould's non-overlapping magisteria (NOMA) (*5*), Dawkins unequivocally concludes: "Darwin made it possible to be an intellectually fulfilled atheist" (*4*).

Dawkins' God
Genes, Memes, and the Meaning of Life
by Alister McGrath
Blackwell, Oxford, 2004. 208 pp. $54.95, £45. ISBN 1-4051-2539-X. Paper, $18.95, £9.99. ISBN 1-4051-2538-1.

Dawkins has generated controversy within the ranks of evolutionary theorists for his strict adherence to Darwinian natural selection ("random mutation plus nonrandom cumulative selection" in his succinct description) as the only mechanism of evolutionary change worth bothering about—Gould called him a "Darwinian fundamentalist" (*6*)—but it is his statements about religion that have drawn attention to him from outside the scientific community. Now, in *Dawkins' God*, we have a book-length analysis by Alister McGrath, professor of historical theology at Oxford. With professional training in the sciences as well as theology (he earned a doctorate in molecular biophysics), McGrath is well qualified to assess Dawkins's literary corpus.

The book begins with an engaging first-person account of McGrath's own journey from atheist to theist, emphasizing the shortcomings of the former and the strengths of the latter (*7*). During his time as a graduate student at Oxford, McGrath began to explore the relation between science and religion, which led him to realize

that Christianity was more sophisticated than his atheism allowed him to appreciate. "While I had been severely critical of Christianity as a young man, I had never extended that same critical evaluation to atheism." When he did, he discovered "that the intellectual case for atheism was rather less substantial than I had supposed." At the same time, he was reading Dawkins, whose conclusions were just the opposite; thus was born this book, decades in the making.

After a brief tour of the life and science of both Darwin and Dawkins, McGrath addresses Dawkins's vision of evolutionary theory as a complete worldview. "I'm a Darwinist because I believe the only alternatives are Lamarckism or God," Dawkins explains, "neither of which does the job as an explanatory principle" (*8*). Because science supports Darwinism, the implications are broad and deep. "The universe we observe has precisely the properties we should expect if there is, at bottom, no design, no purpose, no evil and no good, nothing but blind pitiless indifference" (*9*). What place, then, for God?

The remainder of *Dawkins' God* consists primarily of a point-by-point critique of Dawkins's writings on religion, which McGrath sees as too simplistic and full of easy-to-topple straw men. McGrath summarizes his position thusly: (i) "The scientific method is incapable of adjudicating the God

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hypothesis, either positively or negatively." (ii) "God need not be invoked as an explanatory agent within the evolutionary process" (to be subsequently dismissed). (iii) "The concept of God as 'watchmaker,' which Dawkins spends so much time demolishing, emerged as significant in the eighteenth century, and is not typical of the Christian tradition." This is, in essence, Gould's NOMA—science and religion serve different purposes using different methods, and attempts to bring them into harmony or conflict cannot be logically justified.

Then how do we know there is a God? Faith. According to Dawkins, faith "means blind trust, in the absence of evidence, even in the teeth of evidence" (*10*). This, says McGrath, "bears little relation to any religious (or any other) sense of the word." In its stead McGrath presents the definition of faith by the Anglican theologian W. H. Griffith-Thomas: "It commences with the conviction of the mind based on adequate evidence; it continues in the confidence of the heart or emotions based on conviction, and it is crowned in the consent of the will, by means of which the conviction and confidence are expressed in conduct." Such a definition—which McGrath describes as "typical of any Christian writer"—is an example of what Dawkins, in reference to French postmodernists, calls "continental obscurantism." Most of it describes the psychology of belief. The only clause of relevance to a scientist is "adequate evidence," which raises the follow-up question, "Is there?"

Obviously McGrath must think there is, but he never says. On this point I found the book frustrating. As McGrath's relentless deconstruction of Dawkins unfolds, he repeats, over and over, that religion offers a worldview every bit as sophisticated and worthy of respect as science. His defense of religious faith is a passionate and honorable one, and he demonstrates that some of Dawkins's characterizations of religion are indeed overly simplistic or selective, but he never delivers an answer to the God question. The closest thing to an argument for God's existence I could find in the book is this: "Why should God require an explanation at all? He might just be an 'ultimate,'…one of those things we have to accept as given, and is thus amenable to description, rather than explanation." That may be, but like all other arguments made in favor of God's existence, this only works as a reason to believe *if you already believe*. If you do not already believe, science cannot help you.

I was eager to read *Dawkins' God* because of the gladiatorial weight of the contestants and what they represent. And although McGrath presents many side issues in a pleasantly readable fashion (e.g., Darwin's religiosity, the historiography of science and religion, and how and where religion embraces science), he dodges the biggest question of all, the question at the heart of Dawkins's writings: Is there a God? Whether Dawkins is simplistic or sarcastic or sardonic is a secondary issue. By elevating it to the primary focus of the book, McGrath missed an opportunity to make his case, pace Dawkins, and give us the very best arguments in his arsenal. With McGrath, I still do not know why he believes in God. With Dawkins, there is no doubt about where he stands.

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PLANETARY SCIENCE

Big Book on Our Gas Giant

Linda Rowan

Besides being the largest, most massive, and fastest rotating of the solar system's planets, Jupiter also possesses the strongest magnetic field and most satellites (over 60). Three decades ago, a comprehensive survey of our understanding of the jovian system was published in *Jupiter: Studies of the Interior, Atmosphere, Magnetosphere, and Satellites*, edited by Tom Gehrels, (University of Arizona Press, Tucson, 1976). Now Fran Bagenal, Timothy Dowling, and William McKinnon have joined with 123 other experts to produce *Jupiter: The Planet, Satellites and Magnetosphere*. Developed in the successful format of the earlier volume, this exhaustive magnum opus can appropriately be called *Jupiter II*. Such an updated account is long overdue, and the volume will be essential for anyone studying the "king of the planets."

Jupiter
The Planet, Satellites and Magnetosphere
Fran Bagenal, Timothy E. Dowling, and William B. McKinnon, Eds.
Cambridge University Press, Cambridge, 2004. 731 pp. + CD-ROM. $150, £85. ISBN 0-521-81808-7. Cambridge Planetary Science.

The volume is filled with results from six spacecraft flybys—Pioneer 10 (1972), Pioneer 11 (1973), Voyager 1 (1979), Voyager 2 (1979), Ulysses (1992), and Cassini (2002)—and the Galileo mission, which orbited the planet (1995–2003) and delivered a probe that sampled the uppermost atmosphere. The authors also present data and images from Earth-bound observatories and space telescopes (as in the chapter on comet Shoemaker-Levy 9's 1994 impact into Jupiter) and synthesize findings from theoretical models. Readers will be disappointed by the black-and-white graphics used in the book, but thankfully the accompanying CD and Web site (http://dosxx.Colorado.edu/JUPITER) provide all of the figures in their appropriately stunning colors.

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Cloudy all day. This true color mosaic was constructed from images taken by the Cassini spacecraft as it approached Jupiter in December 2000.

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ENVIRONMENT

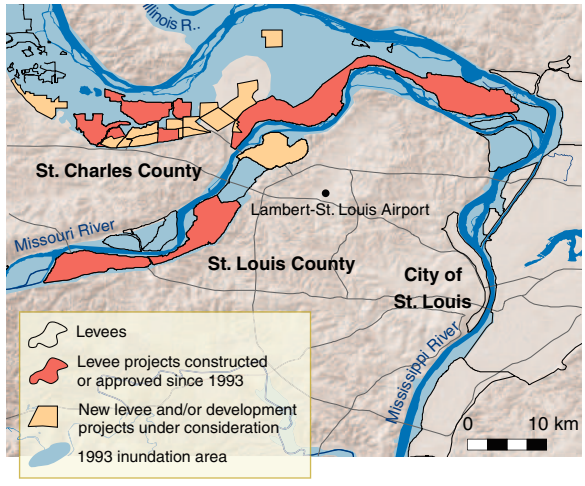
One Step Forward, Two Steps Back on U.S. Floodplains

Nicholas Pinter

The great Midwestern flood of 1993 broke flow records along 1600 km of the Mississippi and Missouri rivers and caused up to $16 billion in damages (*1*, *2*). Formal reviews of U.S. flood-control policy, both before and after the 1993 flood, concluded that the optimum strategy for reducing flood losses is to limit or even reduce infrastructure on floodplains. New emphases on flood-damage prevention included widely publicized Federal Emergency Management Agency (FEMA) buyouts of floodplain properties. In Illinois and Missouri, the two most heavily impacted states, 7700 properties were acquired at a cost of $56.3 million, including the relocation of the town of Valmeyer, Illinois (*3*). Unfortunately, these buyouts are now being massively counterbalanced by new construction on the floodplains. The center of this recent rush onto the floodplain is the St. Louis metropolitan region (see figure, right). This paper explores the impacts of such encroachment, including raising future flood levels, and outlines alternatives that have been proposed and implemented worldwide.

It has been asserted that flood-control structures prevented $19 billion in damages during the 1993 flood; however, most infrastructure on the floodplain would not be there were it not for the historic reliance on levees [e.g. (*4*–*7*)]. Since 1993, the amount of such infrastructure has increased dramatically: 28,000 new homes were built, population increased 23%, and 26.8 km^2 (6630 acres) of commercial and industrial development were added on land that was inundated during the 1993 flood (*8*). In all, $2.2 billion in new development has occurred in the St. Louis area alone on land that was under water in 1993 (*3*).

The majority of this floodplain development has occurred in the state of Missouri, and around St. Louis in particular. Of the total new commercial and industrial development in the 1993 inundation area, 76% was located in Missouri, and 60% in St. Louis and St. Charles counties alone (*8*). Since 1993, projects now complete, under way, and in planning have put or will put 72.8 km^2 (18,000 acres) of the Mississippi and Missouri floodplains near St. Louis behind new levees, enlarged enlarged levees, or floodplain land raised above the 100-year to 500-year protection level (see figure, above). Most of these projects have been financed or heavily subsidized by local governments in each area. The U.S. Army Corps of Engineers also has spent $197 million working on nine local levees in its St. Louis District since 1993 (*3*).



A surge of floodplain development. Levee and floodplain development projects in the greater St. Louis, Missouri, area. This map includes new and enlarged levees and elevation of floodplain land completed since 1993 and development projects under review or proposed. Data sources: levee boundaries and inundation area (*33*), completed projects (*3*), and projects under review, courtesy of Great Rivers Habitat Alliance.

Floodplain development projects in the United States are constrained by FEMA guidelines under the National Flood Insurance Program (NFIP), by wetlands protections specified in the Clean Water Act and administered by the Corps of Engineers, and in some locations by more stringent state and local regulations. The NFIP guidelines limit development in the central portion of the floodplain (the "floodway"), but allow virtually unlimited development across the rest of the floodplain so long as developed areas are either raised above the level of the 100-year flood (the event with a 1% chance of occurring in any year) or protected by levees with at least 100-year protection.

Among the broadest criticisms of flood control by levees is that development in levee-enclosed areas promotes the false expectation that flood risk is reduced to zero. As a National Academy of Science panel concluded, "it is short-sighted and foolish to regard even the most reliable levee system as fail-safe" (*4*). Currently, FEMA removes areas protected by 100-year levees entirely from their flood-hazard maps. Proposals to elevate or protect areas of the floodplain by levees typically must also obtain wetland fill permits from the Army Corps of Engineers under Section 404 of the Clean Water Act. Such permit requests must demonstrate that the project will not unduly impact the "public interest," including adversely affecting flood hazard. In the St. Louis region, requests for wetland fill permits have been granted despite a long history of research documenting adverse effects of levees, including that they have contributed to increased flood levels.

The magnitudes and frequencies of flooding on the Mississippi and Missouri rivers have increased dramatically during the past century [e.g. (*9*–*16*)]. This conclusion has been sidestepped by an often-repeated assertion that "The floods of the Mississippi River Basin are ... acts of God, which man cannot prevent" (*17*). More recently, "… the Great Flood of 1993 ... was not caused by levees, loss of wetlands, navigation structures, flood plain development, or any of several other reasons that have been brought up by various individuals. The flood was caused by unprecedented rainfall" (*18*). That floods are caused by rainfall is self-evident, but this truism camouflages an implication—that various human influences on the river-floodplain system have no impact—that is controverted by extensive research. As the General Accounting Office summarized, "That levees increase flood levels is subject to little disagreement" (*19*). Along the lower Missouri River and the Mississippi River near St. Louis, increases in flood levels of up to 3 to 4 m during the past century have been documented (*12*–*14*).

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Part of the failure to recognize flood magnification owing to levees is because incremental levee expansion projects are evaluated individually, even when many projects are proposed for a given river reach. Uncertainties in modeling relatively small encroachments allow a "fuzzy math" sufficient to assert that each incremental increase in flood levels will be negligible. Corps of Engineers permit regulations state that "Although a particular alteration to a floodplain may constitute a minor change, the cumulative impact of such changes may result in a significant degradation of floodplain values and functions and in increased potential for harm" (*20*). Instead, project permits are being issued on an individual basis, resulting in a "death by a thousand blows" through the incremental loss of floodplain land to development.

To gain a broader sense of whether the surge of floodplain development in Missouri is typical of floodplains across the United States, the Lexis/Nexis full-text database was queried for all references to floodplain development or encroachment. Of 53 major newspapers tracked in the database, 62% of all articles and editorials discussing floodplain encroachment in the past 5 years were in a single newspaper, the *St. Louis Post-Dispatch*. Although St. Louis appears to be the epicenter of the problem, development is overwhelming floodplains in a number of other locations. For example, in Sacramento, California, at least 60,000 new homes and billions of dollars of new infrastructure have been recently built or are planned on several floodplain tracts of the American, Feather, and Sacramento rivers (*21*–*23*). In contrast, other U.S. municipalities—including Denver and Boulder, Colorado; Austin, Texas; Phoenix, Arizona; and Charlotte, North Carolina—have limited encroachment and guided development to more compatible locations and land uses. The explosion of floodplain development around the city of St. Louis and other areas of Missouri appears to be linked to state-level floodplain laws that are among the weakest in the United States. For example, although NFIP guidelines state that no construction in the floodplain should result in more than a 30-cm (1.0-ft.) increase in flood level, other states specify more stringent thresholds. Missouri has passed legislation that prohibits any county from setting any threshold stricter than the 1.0-foot limit (*24*).

The 1982 National Academy of Science report on levees and flood hazard warned that "Adoption by municipal governments of a program of constructing flood control levees raises questions of potential liability for any flood damages that result from improper design or maintenance of such systems" (*4*). A growing body of precedents, including two cases in California during the past year (*25*, *26*), have held municipal, county, and state governments liable for flood damages where those governments encouraged floodplain encroachment or managed flood-control systems that altered natural flooding patterns. Levee failures have been responsible for roughly one-third of all flood disasters in the United States (*4*), and these damages would have been avoided if different floodplain management decisions had been made at the onset (*27*).

Alternatives can be found to the heavy reliance in the United States on structural flood-control measures. In Europe, following severe flooding on the Rhine River in 1993 and 1995, the Dutch government has dramatically shifted its approach to flood control to a policy of "more room for the rivers," meaning creating new storage and conveyance space rather than indulging in new rounds of levee raising (*28*, *29*). On the Meuse River, France, Germany, Belgium, Luxembourg, and the Netherlands adopted the Meuse High Water Action Plan, focused on "land use activities from a water perspective," longer storage and slower release," and "space for the river" (*30*). These programs are not merely theoretical proposals. Since 1988, the Integriertes Rheinprogramm of the state of Baden-Württemberg, Germany, has reduced peak flood stages to 1950 levels by adding 212 million m^3 of storage on the floodplain (*31*). In the Netherlands, the "Room for the Rhine" doctrine was adopted in 1997, and the Dutch government has committed $3 billion to a broad toolbox of levee alternatives (*29*, *30*). In the United States, a blueprint for floodplain management called "No Adverse Impact" has been developed by the Association of State Floodplain Managers, in which "the action of one property owner or community [should] not adversely affect the flood risks for other properties or communities" (*32*).

Thanks to Federal guidelines, buyouts, and enlightened management in many localities, successes in managing U.S. floodplains outnumber the failures. The problem is that when these measures succumb to local economic self-interest and political pressure, small local failures—like cracks in levees themselves—allow massive increases in floodplain infrastructure that can rob the nation of all the net improvements painstakingly won elsewhere. In spite of the lessons learned during the 1993 flood, the St. Louis region and selected other localities across the United States are seeing their floodplains disappear behind new and enlarged levees and under new urban and suburban development.

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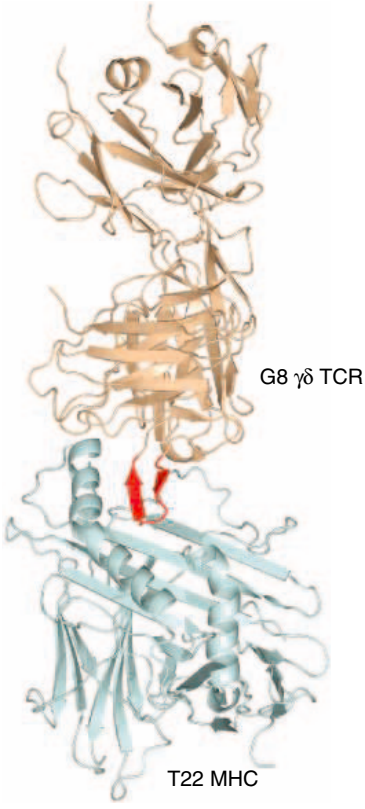
STRUCTURAL BIOLOGY

"D" Is Not for Diversity

David N. Garboczi

There are three types of receptors that bind to antigens—B cell receptors (antibodies), T cell receptors (TCRs) expressed by αβ T cells, and TCRs expressed by γδ T cells. The antigen-binding sites of these receptors result from the recombination of variable (V), diversity (D), and joining (J) gene segments. Because antigens from the microbial world have extremely diverse chemical structures, the range of binding-site specificities must also be very large. All three kinds of antigen receptor have the potential to form an enormous number of antigen-binding sites (*1*). For antibodies and αβ TCRs, this is borne out by their capacity to respond to almost any antigen or antigenic peptide bound to major histocompatibility complex (MHC) molecules. For γδ TCRs, however, few ligands have been identified and clear functions for T cells that bear γδ TCRs remain to be elucidated. Two papers in this issue, by Shin *et al*. (*2*) on page 252 and Adams *et al.* (*3*) on page 227, shed light on the elusive γδ TCRs and their ligands.

From the crystal structures of antibody-antigen and TCR/MHC complexes, we have a broad structural picture of how the diverse amino acid sequences of the receptors are translated into a panoply of antigen-binding sites. However, our structural understanding of γδ TCRs is limited because, until now, the only structures available have been those of a Vδ domain from a human γδ TCR (*4*) and a γδ TCR expressed by human γδ T cells in the bloodstream (*5*). These structures reveal the domains of γδ TCRs and a putative antigen-binding site, but not how a γδ TCR binds to its antigen. Unlike antibodies and αβ TCRs, γδ TCRs have few known ligands. For a molecular understanding of γδ T cell function, we need to know the antigens that γδ TCRs bind and how they are recognized. This requires determining the structure of a γδ TCR in a complex with its antigen. The best-known antigens for mouse γδ TCRs are the nonclassical MHC proteins known as T10 and its close relative T22 (*6*). Now, Shin *et al*. (*2*) demonstrate that there is a substantial population of mouse γδ T cells bearing surface TCRs that carry a full-length D gene segment (Dδ2) and bind to the T22 molecule. In a complementary study, Adams *et al*. (*3*) determine the crystal structure of a γδ TCR/T22 complex. Their structure reveals how G8, one of the γδ TCRs studied by Shin *et al.*, binds to the T22 molecule—G8 uses amino acid residues encoded by the Dδ2 gene.



Unlike αβ TCRs that recognize and bind to antigenic peptides complexed with MHC molecules, γδ TCRs do not recognize peptides. In fact, their recognition of T22 molecules does not depend on the cellular antigen-processing machinery that generates antigenic peptides. For example, T22-reactive γδ TCRs recognize T22 expressed on the surface of insect cells, which have no antigen-processing capability (*7*), as well as T22 molecules produced in bacterial cells and renatured without a trace of peptides (*8*). In their study, Shin *et al*. used fluorescent tetramers of renatured T22 as a staining reagent to sort T22-specific γδ T cells and to clone individual γδ T cells. Tetramers of MHC molecules bind more strongly to T cells (with a higher avidity) than do MHC monomers. Coupled to a fluorescent compound, MHC tetramers enable the specific, but weak, binding between MHC and TCR to be exploited to stain particular T cells. Shin *et al*. also used the release of bound T22 tetramers over time [tetramer decay (*9*)] to measure the binding affinities between several γδ TCRs and T22. Their results agree with the high affinity measured by the surface plasmon resonance technique (dissociation constant $K_D = 0.10$ μM) (*8*) between G8 and T22.

Depending on the region of the body where the γδ T cells are located, their TCRs are encoded by different Vγ and Vδ gene segments (*1*). For example, γδ T cells from mouse spleen express Vγ1 and Vγ4, but γδ T cells in mouse intestine principally express Vγ7. Since these anatomically restricted T cell subsets were discovered, it has been thought that T cell specificity might correlate with V gene segment usage, although the sequence requirements for any class of γδ TCRs to bind to specific antigen remain unclear. When Shin *et al*. sequenced the γ and δ chains of TCRs from γδ T cells retrieved with T22 tetramers, they found that the complementarity determining region–3 loops of the δ subunits (CDR3δ) of the TCRs shared a sequence motif regardless of the Vγ or Vδ gene segments that were rearranged. The motif is composed of amino acid residues encoded by the largely intact Dδ2 gene. It seems that during the gene rearrangements of γδ TCRs, a full-length Dδ2 gene is frequently used to assemble a CDR3δ, explaining the relatively high frequency of T22-specific clones. If other antigens are recognized through similar means using other Dδ2 reading frames or the other mouse Dδ gene, Dδ1, the number of antigens recognized by γδ TCRs may be as few as the number of D-gene reading frames.

New insights into the γδ TCR. (**Top**) The γδ TCR (brown) of the mouse G8 γδ T cell clone binds to T22 (blue), a nonclassical MHC molecule, primarily through its CDR3δ loop (red). The G8 γδ TCR seems to hang off the edge of T22 by means of the CDR3δ loop that anchors it to the floor of a cavity in the T22 MHC-like molecule [see also figure 1 in (*3*)]. (**Bottom**) The amino acid sequence used by the G8 γδ TCR to bind to T22 includes the full-length diversity (D) gene segment, Dδ2, which encodes the SEGYE sequence. A large number of γδ T cells make use of a full-length Dδ2 gene to form part of their CDR3 loop. Most of the T cells that bind to the T22 molecule use the reading frame that encodes the sequence SEGYE.

In their study, Adams *et al*. (*3*) present the crystal structure of the G8 γδ TCR bound to the T22 molecule. In this first look at a γδ TCR bound to

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its ligand, Adams *et al*. reveal an unusual (by αβ TCR precedents) arrangement of the receptor with the MHC-like T22 protein. With αβ TCRs, these receptors approach the top of the peptide/MHC complex and use most of their six CDR loops to make many contacts with peptide and MHC protein. In the case of the G8 γδ TCR, this receptor seems to hang off one side of the MHC-like T22 protein, making contacts with T22 mostly with its CDR3δ loop (see the figure). The loop, containing amino acid residues encoded by Dδ2, fits into the (nonfunctional) peptide-binding site of T22, with the Dδ2 residues (SEGYE) doing most of the binding. Adams *et al*. also show that the G8/T22 complex forms a dimer in the crystal that may be biologically relevant. This is the first time that TCR/MHC complexes in a crystal have been shown to form dimers that could be reasonably positioned on the T cell surface (although the actual existence of dimers on the T cell surface awaits further confirmation).

Although γδ T cells have a large potential sequence diversity stemming from the many possible rearrangements of their TCRs, so far they have not been shown to make use of that diversity. Their recognition of a limited number of ligands appears to constrain γδ TCR sequence diversity. As shown in these two studies, the full-length Dδ2 gene segment translated in one of its open reading frames encodes the binding residues for T22 molecules. The binding activities that other D gene segments and their reading frames may encode would provide clues to other γδ T cell ligands and may help to elucidate the functions of these enigmatic cells.

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ASTRONOMY

A Stellar Swan-Song

Martin Asplund

The time scales on which stars normally evolve are extremely long. The Sun was born ~4.5 billion years ago and is only halfway through its evolution. More massive stars evolve faster, but still require millions of years to complete their life cycles. However, some phases of stellar evolution are very rapid. Arguably the fastest case of stellar evolution known (other than complete stellar explosions, such as supernovae) is the rebirth of a giant star.

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In such an event, a hot stellar ember (called a white dwarf) about the size of Earth balloons within a few years to become a cool giant star some 100 times the size of the Sun. These events are observed very rarely. Only three such stars—FG Sagittae, V605 Aquilae, and, most recently, Sakurai's Object (V4334 Sgr)—have ever been observed during the act. On page 231 in this issue, Hajduk *et al*. (*1*) report that Sakurai's Object has started to heat up and contract again, only ~10 years after it first swelled up to gigantic proportions.

While hunting for comets in February 1996, the Japanese amateur astronomer Yukio Sakurai noticed a new starlike object in the constellation Sagittarius. The object was initially thought to be a nova, but inspection of its spectrum and the fact that the object did not fade with time soon revealed that it was a born-again giant star (*2*). Astronomers have studied its evolution ever since. Its stellar surface cooled from ~8000 K in 1996 to ~6000 K within a year (*3*, *4*). Over the same period, its chemical composition changed markedly, with a decrease in hydrogen and an increase in lithium and some heavy elements (*5*). The star also developed a dense wind in which dust condensed, obscuring our view of the star (*6*, *7*). Today, the dust is so impenetrable that it renders the star practically invisible in optical light.

The remarkable behavior of Sakurai's Object is thought to result from the revival of nuclear burning in a white dwarf. Toward the end of their lives, stars with an initial mass of about 1 to 8 solar masses expand to giant dimensions and become so-called asymptotic giant branch (AGB) stars (*8*). Their nuclear energy is produced alternately by helium- and hydrogen-burning in shells around the stellar core. Each ignition of helium-burning is sudden and occurs when enough helium (which is produced by hydrogen-burning) has accumulated. Such helium flashes happen every ~10,000 to 100,000 years in AGB stars. Eventually, the entire outer stellar envelope is ejected to form a planetary nebula, while the inner parts contract to form a white dwarf, which is no longer capable of nuclear burning.

In some cases, however, the star can temporarily avoid fading to stellar oblivion. Because of the temperature increase in the stellar interior resulting from the contraction, a final helium flash may occur when the star has long left the AGB phase. The reignition of nuclear burning releases energy, which causes a rapid expansion and cooling of the stellar surface; the rejuvenated star appears as an AGB star for the second time (see the figure) (*9*).

The observed compositional changes in Sakurai's Object were caused by mixing of the outer layers with nuclear-processed gas from the interior. This mixing has dredged up previously burnt material and has ignited fresh hydrogen-burning and other associated nuclear reactions, such as lithium production and neutron capture processing. Because the observed decrease in hydrogen content reflects the depletion of the stellar nuclear fuel, born-again giants provide a unique opportunity to study stellar nucleosynthesis and convection directly. The born-again phase lasts only 10 to 1000 years before the star retraces its earlier evolution to the white dwarf regime, and this time there is no turning back. The brevity of this phase explains why the phenomenon is so rarely observed, even though 20% of all AGB stars are predicted to go through this phase (*10*).

Because of the faintness of the star, the evidence presented by Hajduk *et al*. that Sakurai's Object has started to heat up again is indirect. Their observations reveal a new radio-emitting region inside the old planetary nebula. The photo-ionization responsible for this newly ionized gas requires stellar surface temperatures of >20,000 K, much higher than those observed in the late 1990s. Previous observations of optical nebular emission from the immediate surroundings of the star had suggested the same, but the findings were inconclusive (*11*). Both the radio and optical emissions suggest an asymmetric structure, similar to that of V605 Aquilae, A30, and A78, which are believed to have been born-again giants in the recent past (*12*). This structure may indicate that the gas escapes the star preferentially in two opposing directions. We appear to be witnessing the nascent stages of the formation of a new planetary nebula in Sakurai's Object, something that has never been observed before.

Sakurai's Object has evolved much faster than suggested by previous stellar modeling of born-again giant events. Hajduk *et al.* (*1*) present evolutionary calculations in which

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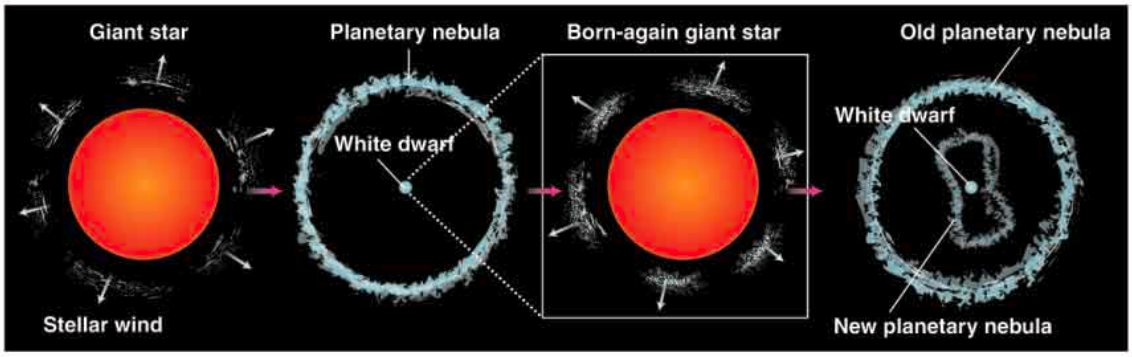
convective mixing efficiency is reduced as a result of the rapid nuclear burning. Because hydrogen burning then occurs closer to the surface, stellar evolution is accelerated, in agreement with observations (*13*, *14*). The model results can explain the gross properties and the observed variations in the chemical composition of Sakurai's Object, including the production of lithium and the low carbon isotopic ratio ($^{12}C/^{13}C \approx 4$). Hajduk *et al.* even argue that born-again giants may be the dominant source of ^{13}C in the Universe. Indeed, certain presolar grains in primitive meteorites found on Earth may come from born-again giants (*15*).



Stellar reignition. Toward the end of its life, a star like the Sun swells up to become a cool giant star (**left**). Eventually, the outer envelope is ejected and forms a planetary nebula. With nuclear-burning having ceased, the inner parts contract to become a hot white dwarf (**second from left**). However, a final flash of helium-burning may reinflate the star back to giant dimensions, a 10,000-fold increase in radius (**second from right**). Because the star rapidly runs out of nuclear fuel, this born-again giant phase lasts for only 10 to 1000 years. Soon, a new planetary nebula is formed, while the star retraces its earlier evolution toward the white dwarf regime (**right**).

Given the unprecedented pace with which Sakurai's Object has evolved over the past decade, its future behavior will be scrutinized closely. As the star heats up, it will eventually destroy its obscuring cocoon of dust and become visible again inside the new planetary nebula. Studies of its chemical composition would then be high on the agenda. The real-time evolution of Sakurai's Object is likely to continue to unfold in the coming decades, to the delight of astronomers.

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CELL BIOLOGY

GSK-3β and Microtubule Assembly in Axons

Feng-Quan Zhou and William D. Snider

Microtubules are the principal cytoskeletal components of axons, the long processes that link the excitable cells (neurons) of the nervous system. Thus, control of microtubule polymerization and stability is a key regulatory step in axon growth during development and regeneration after nerve injury. Moreover, the regulation of these two processes is an important target of both growth-promoting and growth-inhibiting extracellular signals.

Microtubules are linear structures composed of 13 protofilaments assembled from α and β tubulin heterodimers. Because the α/β tubulin dimers are organized in a head-to-tail fashion, the microtubules are polarized structures with a "minus" end, from which microtubules are nucleated, and a "plus" end, where polymerization of tubulin dimers occurs. The microtubule plus ends cycle between phases of growth and shrinkage, a property termed dynamic instability (*1*). The net growth of microtubule polymers is determined by the rate of dimer addition and the time that the plus ends spend in the growth phase. A major recent advance has been the discovery of proteins that bind to tubulin dimers or microtubule plus ends and regulate microtubule polymerization and stability (*2*). Although these proteins are under intensive investigation in nonneuronal cells, we still know little about how they regulate microtubule assembly during axon growth and regeneration. In particular, we are still missing links between well-studied axon growth-promoting and axon growth-inhibiting extracellular signals and the regulation of microtubule assembly.

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Two papers recently published in *Cell* (*3*, *4*), together with other recent work (*5*), suggest that glycogen synthase kinase 3β (GSK-3β) is a crucial player in the regulation of axon morphogenesis downstream of phosphatidylinositol 3-kinase (PI3K) signaling. First purified in 1980, GSK-3β is a serine/threonine kinase that mediates the inactivation of glycogen synthase. Surprisingly, GSK-3β has emerged as a key regulatory kinase in the nervous system with involvement in processes ranging from neural development to mood stabilization to neurodegeneration (*6*).

Unlike many other kinases, GSK-3β is usually active in resting cells. Upon activation of the PI3K signaling pathway, GSK-3β is inactivated through phosphorylation of the serine-9 residue in its amino-terminal region (*7*). In the two new studies, Jiang *et al*. (*3*) and Yoshimura *et al*. (*4*) show that specification of rapidly elongating axonal processes versus more slowly growing dendritic processes in hippocampal neurons is regulated by GSK-3β activity. Spatially localized inactivation of GSK-3β by PI3K in a single immature neurite was necessary for future axon specification and maintenance, whereas global inactivation of GSK-3β led to the formation of multiple axons. Remarkably, inhibition of GSK-3β activity

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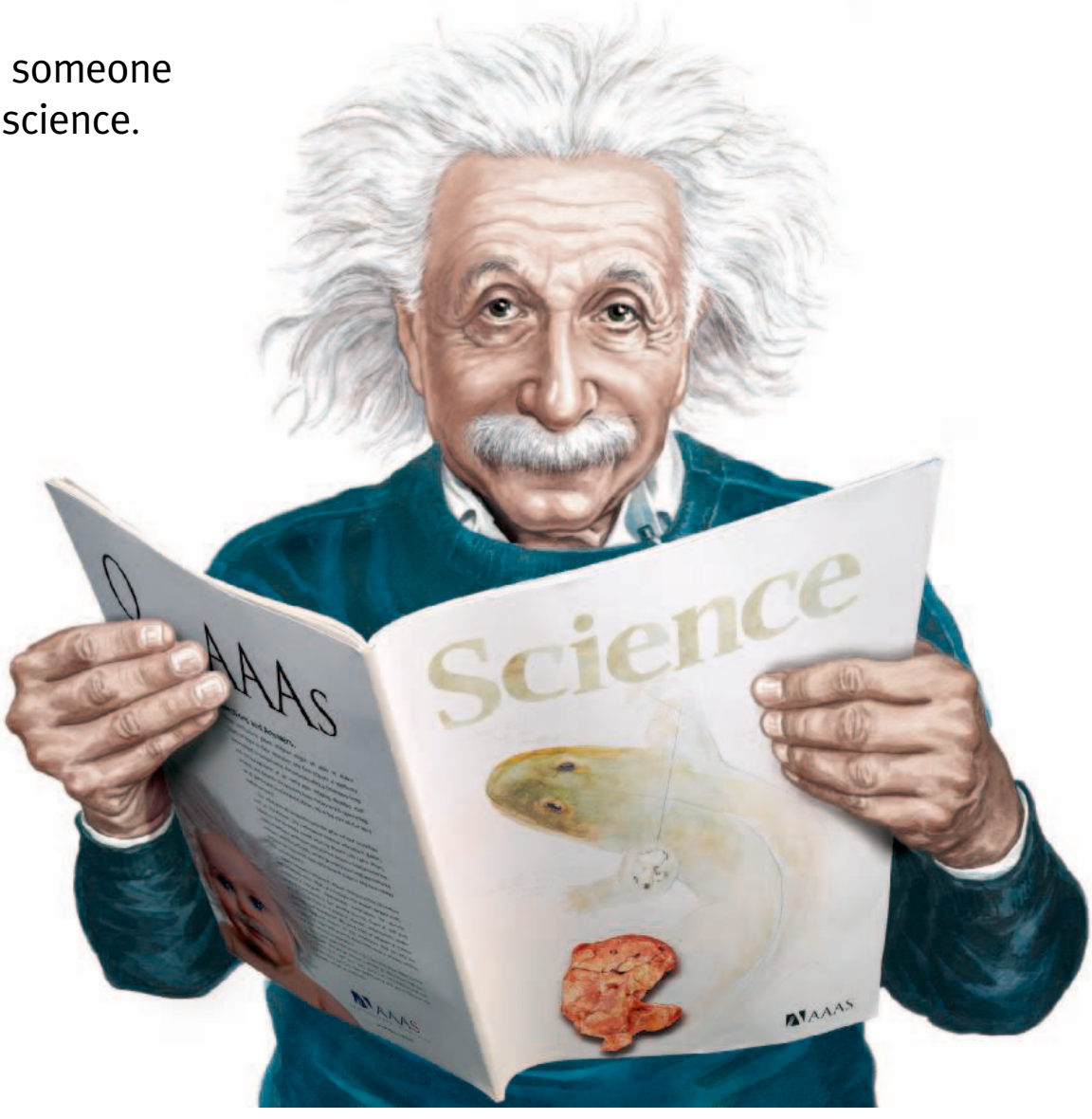

Albert Einstein
1879–1955

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with specific kinase inhibitors was even able to turn mature dendrites into axons (*3*). Yoshimura *et al*. also showed that hippocampal axon growth triggered by neurotrophin stimulation was dependent on GSK-3β inactivation. These latter findings are in line with previous work in dorsal root ganglion neurons showing that localized inactivation of GSK-3β at the growth cone is required for rapid axon elongation induced by nerve growth factor (*5*).

The ability of GSK-3β to control axon morphogenesis presumably relies on its ability to regulate a number of different microtubule binding proteins (MBPs). A fascinating picture is emerging in which these different MBPs may regulate specific aspects of microtubule assembly. For example, Fukata *et al*. (*8*) recently demonstrated that collapsin response mediator protein 2 (CRMP-2) binds to α/β tubulin dimers and thus regulates microtubule polymerization. The ability of CRMP-2 to bind to tubulin dimers is abolished when it is phosphorylated by GSK-3β (*4*). Thus, inactivation of GSK-3β results in CRMP-2 dephosphorylation, which leads to enhanced microtubule polymerization and axon growth. Indeed, hippocampal neurons induced to express a mutant version of CRMP-2 that cannot be phosphorylated by GSK-3β formed multiple axons.

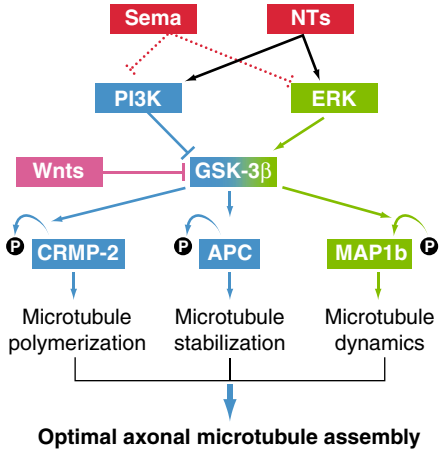
Another MBP regulated by GSK-3β is adenomatous polyposis coli (APC), a tumor suppressor protein first identified as the product of the gene mutated in familial adenomatous polyposis (characterized by a predisposition to developing intestinal polyps and colon cancer) (*9*). This MBP then was found to belong to a group of proteins that copolymerize with microtubules at their plus ends, termed plus end tracking proteins (+TIPS). These +TIPS promote microtubule stabilization by increasing the time that microtubules spend in the growth phase (*2*). Similar to CRMP-2, phosphorylation of APC by GSK-3β prevents its ability to bind to microtubules (*10*). GSK-3β inactivation induced by nerve growth factor via the PI3K signaling pathway enhances APC-microtubule interactions, which is functionally necessary for the axonal growth induced by nerve growth factor (*5*). Taken together, these studies indicate that inactivation of GSK-3β downstream of PI3K controls both microtubule polymerization and stabilization via the simultaneous regulation of multiple MBPs.

MAP1b is another well-known target of GSK-3β. There is evidence that phosphorylated MAP1b destabilizes microtubules and maintains them in a dynamic state (*11*). The dynamic property of microtubules (which cycle between growth and shrinkage) enables them to efficiently probe the intracellular space and respond to extracellular signals. Inhibiting microtubule dynamics in neurons even without affecting microtubule polymerization retards axon growth and impairs axon guidance and branching in response to guidance cues (*1*). In marked contrast to APC and CRMP-2, MAP1b is activated by GSK-3β phosphorylation. Inhibition of GSK-3β activity abolishes MAP1b phosphorylation and reduces microtubule dynamics. Interestingly, phosphorylated MAP1b is also found at the distal ends of growing axons, thus colocalizing with dephosphorylated APC and CRMP-2 (*11*).

How can GSK-3β be active toward some substrates and inactive toward others at the same time and in the same place? The answer may lie in how GSK-3β recognizes and phosphorylates its substrates. A special feature of GSK-3β is that it phosphorylates substrates that have been "primed" by other kinases. The kinase activity of GSK-3β toward these "primed" substrates is abolished when its amino-terminal serine-9 residue is phosphorylated by kinases downstream of PI3K. Both APC and CRMP-2 are examples of "primed" substrates (*4*, *10*). However, there is emerging evidence that serine-9 phosphorylation has little effect on GSK-3β activity toward substrates that do not require "priming" (*7*). MAP1b is thought to be one such "unprimed" substrate (*11*). Thus, GSK-3β may phosphorylate MAP1b and maintain microtubule dynamics even when it is phosphorylated at serine-9. Interestingly, the different activities of GSK-3β toward primed versus unprimed substrates may be regulated by distinct signaling mediators. Accumulating evidence shows that the activity of GSK-3β toward its primed substrates, such as APC and CRMP-2, is blocked by PI3K. Studies from Gordon-Weeks's group suggest that the activity of GSK-3β toward MAP1b is increased via the activation of extracellular signal–regulated kinase (ERK) (*12*).

In addition to neurotrophins, other extracellular factors that enhance axon growth or attract axons, such as Wnts and Netrin, affect GSK-3β activity. However, these signals regulate GSK-3β activity via distinct pathways. For instance, Wnt proteins that attract growing axons (*13*) and enhance axon branching (*14*) inactivate GSK-3β (independently of serine-9 phosphorylation) through their Frizzled receptors (*7*). Netrin, on the other hand, acts in a similar way to neurotrophins, regulating GSK-3β activity through serine-9 phosphorylation (*15*) (and also regulating GSK-3β activity toward MAP1b). Moreover, GSK-3β is also an important regulator of Hedgehog signaling, yet another pathway that influences axon guidance (*16*). For all of these pathways, it will be important to investigate the part played by MBPs downstream of GSK-3β in mediating the morphological response of neurons.

Factors that inhibit or repel axon growth, such as semaphorin 3A, activate GSK-3β by inhibiting serine-9 phosphorylation, thereby constituting a particularly important regulatory mechanism. Thus, the localization of serine-9 phosphorylation in the growth cones of dorsal root ganglion neurons observed in the presence of nerve growth factor is abolished by addition of nanomolar concentrations of semaphorin 3A (*17*). One study suggests that semaphorins antagonize neurotrophin signaling (*18*). Activation of semaphorin signaling and subsequent GSK-3β activation result in phosphorylation and inactivation of CRMP-2, which then impairs microtubule polymerization (*19*). Taking all of these findings together, it seems that GSK-3β may be a critical node where multiple extracellular cues converge to regulate axon morphogenesis. Growth-promoting activities result in phosphorylation of GSK-3β at



A three-pronged assembly task for GSK-3β. GSK-3β regulates all three aspects of microtubule assembly in axons. To promote axon assembly, neurotrophins inactivate GSK-3β by phosphorylation of serine-9 via the PI3K signaling pathway. Phosphorylation of GSK-3β allows dephosphorylation of CRMP-2 and APC, which in turn promote microtubule polymerization and stabilization. Neurotrophins also enhance the activity of GSK-3β toward MAP1b (independent of serine-9 phosphorylation) via the ERK pathway. Phosphorylation of MAP1b ensures maintenance of microtubule dynamics. Coordinated regulation of microtubule polymerization, stabilization, and dynamics ensures optimal microtubule assembly in axons. Wnt proteins inactivate GSK-3β via mechanisms independent of serine-9 phosphorylation and may regulate some of the same MBPs to control axon morphogenesis. Extracellular cues that repel axon growth, such as semaphorin, may regulate GSK-3β activity in the direction opposite to that of neurotrophins, resulting in axon repulsion.

serine-9 or inactivation via other signaling pathways. In contrast, growth-inhibitory signals act in the opposite direction to dephosphorylate the serine-9 residue.

All of these results suggest a potential strategy for promoting axonal regeneration after neural injury using pharmacological inhibitors of GSK-3β. Indeed, we have recently demonstrated that inactivation of GSK-3β is necessary for axon regeneration from mature neurons of the peripheral nervous system after nerve transection (*20*). However, it must be emphasized that efficient axon extension probably requires localized inactivation of GSK-3β at the distal end of the growing axon, as well as activity of GSK-3 toward "unprimed" substrates. Although low concentrations of GSK-3β inhibitors lead to elaboration of multiple axons by hippocampal neurons, more complete global inhibition of GSK-3β almost certainly impedes axon growth (*5*, *14*). Thus, manipulation of GSK-3β may be a promising therapeutic strategy to promote functional recovery after injury to the central nervous system. But an important challenge remains how to achieve appropriate local regulation of GSK-3β using pharmacological inhibitors or gene therapeutic constructs.

GSK-3β may be a "master" kinase that mediates convergent signals from multiple extracellular cues affecting axon morphogenesis. It achieves this by coordinating regulation of all three aspects of microtubule assembly: polymerization, stabilization, and dynamics (see the figure). However, regarding the functions of GSK-3β in regulating neuronal morphology, its control of microtubule assembly may only be the "tip of the iceberg." GSK-3β almost certainly regulates actin filaments, the other important component of the axonal cytoskeleton (*17*). Furthermore, GSK-3β presumably regulates metabolism required for axon growth both locally in the axon and at the level of gene transcription by controlling the NFAT (nuclear factor of activated T cells) family of transcription factors and possibly other transcriptional regulators. Surprisingly, in view of its importance, the nervous system of mice that lack GSK-3β has not been extensively analyzed, and no animals lacking the related isoform, GSK-3α, have yet been reported.

Developing inhibitors of GSK-3β is a major target of the pharmaceutical industry because of the potential of such drugs for treating type 2 diabetes and Alzheimer's disease. The success of lithium in treating mood disorders may reflect its ability to inhibit GSK-3β, among other effects (*6*). Given the evidence that GSK-3β is important for regulating nervous system development, the use of GSK-3β inhibitors during pregnancy will need to be very carefully evaluated.

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PSYCHOLOGY

Infants' Insight into the Mind: How Deep?

Josef Perner and Ted Ruffman

Although primates and other animals seem to have some understanding of mind (that is, the behavior of others), the concept of belief seems to be a specifically human ability. Comprehending false belief is the clearest sign of understanding a critical aspect of the mind: its subjectivity and its susceptibility to manipulation by information. It is thought that children develop an understanding of false belief around 4 years of age. However, on page 255 of this issue, Onishi and Baillargeon (*1*) report that infants as young as 15 months have insight into whether a person acts on the basis of a mistaken view (false belief) about the world. This discrepancy touches on important issues. An understanding of false belief at 4 years of age suggests that this ability may be constructed in a cultural process tied to language acquisition. In contrast, competence at 15 months suggests that this ability is part of our purely biological inheritance. What could account for the discrepant findings?

Children's understanding of false belief has hitherto been assessed using a verbal false-belief task in which the experimenter enacts stories. An example of such a story is as follows: A protagonist (let's call him Max) puts a toy or doll (object) in one location and then doesn't see it moved to a second location (*2*). When asked by the experimenter, most 3-year-olds wrongly claim that Max will look for the object in the second location (where they know it is). This finding with 3-year-olds has been confirmed despite many attempts to improve the potential shortcomings of the verbal false-belief task [see meta-analysis by Wellman *et al.* (*3*)]. These results contrast with those from Onishi and Baillargeon's study in which 15-month-old infants were tested with a nonverbal false-belief test. In this test, infants were familiarized with an adult actor hiding and then retrieving a toy (a plastic slice of water melon) in either a yellow or a green box (see the figure). The looking times of the infant subjects were then computed in a series of trials that tested whether the actor held a true or false belief about the location of the toy. Onishi and Baillargeon found that the infants "expected" the actor to search for the toy based on the actor's belief about its location, regardless of whether the location was actually correct. So, why would 3-year-olds fail to provide the correct answer in a verbal false-belief test, when 15-month-old infants can correctly anticipate erroneous actions in the nonverbal false-belief test?

Part of the explanation might come from previous studies that used eye gaze as a measure of understanding in 3-year-olds. Three-year-olds look to the correct (initial) location when anticipating Max's return there, even when they explicitly make the incorrect claim that Max will go to the second location. This early indication of understanding Max's mistake has been dubbed implicit, because many of these children show no awareness of the knowledge implicitly conveyed in their correct eye gaze (*4*). Nonetheless, children at the age of 2½ years show absolutely no sign of this earlier, implicit understanding (*5*).

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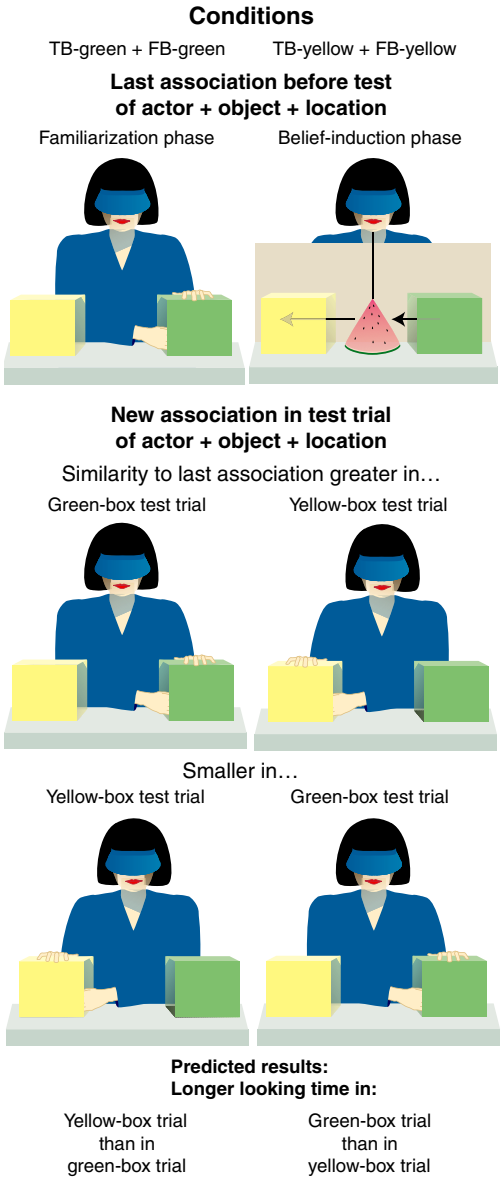

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Now you see it...now you don't. Reanalysis of Onishi and Baillargeon's experimental conditions in a nonverbal false-belief task (*1*). The left column shows the critical events for test trial conditions in which infants looked longer in the yellow-box than in the green-box test trials. These two conditions have in common that the last appearance of the adult actor was during the familiarization phase, where the actor was grasping the object in the green box ("actor-object-green box" association). This makes it possible that the looking time by the infant subjects was shorter in the green-box test trials—as reported (*1*)—because the actor-object-location combination in this test trial is more similar to the last seen association of these three elements than in the yellow-box test trial. The right column shows the same results for the other two conditions, in which looking time for test trials was reversed. This can be explained by the fact that the last appearance of the adult actor was during the belief-induction phase, where the actor observed the object disappear into the yellow box ("actor-object-yellow box" association). Hence, under these conditions, infants found the yellow-box test trials more similar to the last actor-object-location combination than the green box trials, which explains the reversal in looking times. (TB, true belief; FB; false belief)

Converging evidence comes from children's word learning, which also shows sensitivity to false belief around 3 years and not before (*6*). In sum, the evidence of an earlier, implicit understanding does not solve but rather exacerbates the puzzle about Onishi and Baillargeon's finding with infants: Where would the implicit understanding be hiding between 15 months and 3 years?

By adopting particular assumptions about how infants encode events and behavior, we propose two explanations for the apparent early competence of infants that imply an evolutionary, innate bias for understanding the mind. Infants encode events and behavior the way they do because this encoding captures something useful about how people tend to act only because people are endowed with minds. Yet there is no need to assume an understanding on the infant's part that a mind mediates a particular behavior.

Our first account of Onishi and Baillargeon's data is based on neuronal activation as babies process the events of the nonverbal false-belief task (see the figure). Our suggestion is that babies create three-way actor-object-location associations. During the familiarization phase of the test, an adult actor watched by the infants last observes the object (water melon slice) in the yellow box under two conditions (right column) and in the green box under another two conditions (left column). Neurons remember this information both in an active manner (through sustained firing in the prefrontal cortex) and in a latent manner (through altered firing thresholds in nonfrontal regions) (*7*). If an association of elements "actor-object-yellow box" is still sustained in the frontal cortex when babies are exposed to the test stimuli, a consistent test combination will need less processing and, consequently, a shorter "looking" time than a new combination of elements (e.g., actor-object-green box). In the latter case, babies might need longer looking times because, when they examine the new combination, they must form a new association.

A similar increase in looking times may also stem from changes in latent activation in nonfrontal regions, where neurons code for the recency of exposure and increase their firing when a nonrecent stimulus is presented. Even rats are able to represent new arrangements of three familiar stimuli, resulting in increased neuronal activation in the postrhinal cortex and hippocampus and less activation in the dentate gyrus and subiculum (*8*). New arrangements of "actor-object-location (yellow box versus green box)" could result in longer observation times because of the differential activation of neurons that code for the recency of stimuli. Both of these explanations have a clear testable prediction that differs from explanations based on understanding belief: The actor's intentional search for the object in a box in the test phase is not critical. The actor could do something equally interesting (but nonintentional) at either box, and this would also result in the same pattern of differential looking in infants.

For our second account of Onishi and Baillargeon's findings, we acknowledge their suggestion that infants expect the observed person to act in a particular way. However, we propose that this can be based on behavior rules. Infants may have noticed (or are innately predisposed to assume) that people look for an object where they last saw it and not necessarily where the object actually is. Again, such a rule captures something implicit about the mind, because the rule only applies as a result of the mind mediating between seeing and acting. Nonetheless, infants can simply know the rule without any conception that the mind is the mediator. For instance, O'Neill (*9*) found that when requesting an object, 2-year-olds gesture more to the object's location if a parent had not witnessed its placement on a shelf. This finding is compatible with a 2-year-old's understanding of the parent's need for knowledge. It is also equally compatible with a 2-year-old's understanding of the link between the behavior of not having looked at the object in its new location and the likely action of looking in the wrong place (which needs to be prevented by gesturing to the right location).

Povinelli and Vonk (*10*) recently argued that extant evidence for the social intelligence of primates leaves open the question of whether they merely know about behavior or whether they also know about the mental states that mediate behavior. No explanatory power or theoretical parsimony is gained by assuming that animals know about the mind. This criticism also applies to the traditional verbal false-belief task (featuring Max) that children do not pass until 4 years of age. So, it is important to realize that claims about 4-year-olds' understanding of belief cannot be based solely on their positive response to this particular test. The conclusions drawn from the false belief task are warranted only because understanding of false belief around 4 years can be demonstrated in a

variety of belief-inducing situations [in which behavior rules would lead to contradictory predictions of actions (*11*)]. Only the assumption that children acquire an understanding of belief at this point can explain why they start to make correct predictions of actions in these different situations at the same time. Demonstration of such flexible use of belief understanding is missing from studies of both primates and infants (and from studies of the implicit understanding of 3-year-olds).

Assuming that primates have a genetic predisposition to acquiring behavior rules, we can concoct a plausible story about human development. Inheriting from our evolutionary ancestors this predisposition, infants start with a "core theory" (*12*)—that is, knowledge that stays close to the perceptible. Then, children develop a deeper mental understanding of behavior through enculturation into a language community. This contention is supported by increasing evidence that the explicit understanding of belief around 4 years of age strongly relates to language development. Most notably, deaf children raised by hearing parents suffer from a language delay of several years that is also reflected in their late understanding of false beliefs. Thus, we can conclude that the acquisition of our adult theory of mind has a strong evolutionary basis and is deepened by universal aspects of culture, and by linguistic communication in particular.

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CHEMISTRY

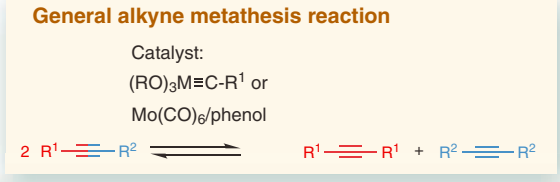
How Are Alkynes Scrambled?

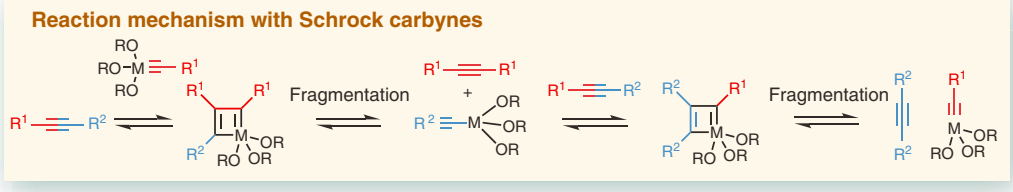
Uwe H. F. Bunz

In a metathesis reaction, two molecules exchange atoms or groups of atoms. For example, two alkynes—hydrocarbons that contain a carbon-carbon triple bond—may swap their substituents (see the first figure, top panel). Such alkyne metathesis reactions, discovered by Mortreux in 1974 (*1*), are very useful for synthesizing complex natural products (*2*), semiconducting polymers, and macrocyclic compounds (*3*, *4*). On page 234 of this issue, Bino *et al.* (*5*) shed light on how some of these reactions are catalyzed.

Mortreux found that treating a disubstituted alkyne with a mixture of two compounds—molybdenum hexacarbonyl [$Mo(CO)_6$] and a phenol derivative called resorcinol—led to the formation of an "in situ" catalyst and yielded a statistical mixture of three alkynes, with their substituents scrambled (*1*). Variants of this catalyst system containing more acidic phenols have been used successfully in the synthesis of polymers and natural products (*1*–*4*, *6*, *7*). However, the chemical nature of the in situ catalysts remains unknown.

In an effort to understand how alkyne metathesis reactions are catalyzed, Schrock and co-workers (*8*, *9*) have produced tungsten and molybdenum carbynes of the type $(RO)_3M{\equiv}C\text{-}CMe_3$, where M is W or Mo and R is Me_3C or aryl (see the first figure, top panel). These complexes, which are air sensitive and are now commercially available, are highly active, structurally defined catalysts for alkyne metathesis. In the case of tungsten carbynes, the reaction proceeds via two cyclic intermediates, which each fragment into a metal carbyne and an alkyne (see the first figure, bottom panel) (*8*, *9*). If one of the product alkynes is removed from the reaction mixture—for example, by distilling off the alkyne with the lower boiling point—the metathesis reaction will yield only the desired metathesis product.





Alkyne metathesis and its mechanism. (**Top**) Two types of compounds—the original Mortreux-type in situ catalysts and the carbynes pioneered by Schrock and co-workers—are known to catalyze alkyne metathesis. (**Bottom**) In the case of carbynes, insights have been gained into the catalytic intermediates. R^1 and R^2 are organic residues, M is tungsten or molybdenum, and RO are bulky alkoxide or aroxide ligands.

Zhang and Moore have obtained variants of the Schrock carbynes (*10*, *11*) by reaction of the molybdenum complex $(R_2N)_3Mo{\equiv}C\text{-}Et$ (*1*, *12*) with 4-nitrophenol. The resulting carbyne, $(O_2N\text{-}C_6H_4\text{-}O)_3Mo{\equiv}C\text{-}Et$, is the most active alkyne metathesis catalyst reported so far and tolerates sulfur atoms adjacent to the catalytic center: It catalyzes the conversion of thiophene-containing monomers into organic semiconductors. This is an important achievement in the field of alkyne metathesis; none of the earlier alkyne metathesis catalysts are able to perform this transformation.

Nevertheless, the simpler Mortreux-type in situ catalysts are powerful for synthetic applications, provided the substrates are sufficiently robust and do not contain nitrogen or sulfur functionalities in close proximity to the alkyne unit. But despite their usefulness, there is no mechanistic understanding of this reaction. It has been suggested that it may proceed via an intermediate resembling the carbyne complexes discussed above, but it remains unclear how these carbynes would form from molybdenum hexacarbonyl and phenols. Bino *et al.* (*5*) bridge this chasm in understanding and shed some light on possible intermediates of alkyne metathesis with in situ catalysts.

Reaction of molybdenum hexacarbonyl

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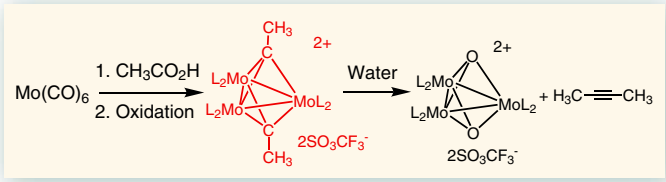
with acetic acid yields a trinuclear molybdenum complex that is stable and can be stored indefinitely (*13*). Bino *et al.* now show that if this species is oxidized in aqueous solution at neutral pH (see the second figure, middle), the carbon atoms in the complex couple to form butyne and a triangular molybdenum cluster containing two oxygen bridges (see the second figure, right) (*1*). When they performed the same reaction in the presence of HBr, they were able to isolate a triangular molybdenum complex with an intact butyne ligand and characterize it with single-crystal x-ray diffraction.



A possible intermediate. In this reaction, reported by Bino *et al.* (*5*), an alkyne is formed stoichiometrically from a trinuclear molybdenum cluster compound. The in situ catalysts of Mortreux-type alkyne metathesis may resemble the structure of the trinuclear molybdenum cluster shown on the right and may proceed via an intermediate similar to that of the cluster shown in red. L may be acetate or water.

These results are important for several reasons. First, the system is sufficiently similar to the in situ alkyne metathesis catalysts to determine how these catalysts might work (*1*–*3*, *6*, *7*). It is surprising that these catalysts may not involve metal-carbon triple-bonded species, but rather trinuclear alkylidyne cluster arrangements. Second, the compound works in water: Upon dissolution in water, the oxidized cluster loses butyne at room temperature. It may therefore be possible to develop an environmentally friendly alkyne metathesis reaction that uses water as the solvent or at least works in the presence of water.

To turn the salts of Bino *et al.* into a synthetically useful metathesis catalyst, the clusters will have to be coaxed to undergo catalytic alkyne metathesis. This could, for example, be done by slow addition of oxidants or by performing the reactions in air at elevated temperatures. Many more parameters can be manipulated to develop a catalytically active system.

What would be the ideal in situ alkyne metathesis system? It should form from a commercially available organometallic or inorganic starting material, to which an activator would be added. The active catalyst would preferably form at room temperature or slightly above and would be stable in the presence of water or oxygen (the latter is easier to exclude if necessary). The metathesis reaction should furnish the product in high yield within a reasonable amount of time. The system would also have to show high functional-group tolerance. The activator should be able to moderate or increase the activity of the catalyst system to a point where it can be manipulated to match the needs of the substrate.

In alkene metathesis, ruthenium-based catalysts (*14*, *15*) are very close to this ideal, but in alkyne metathesis, the catalytic "philosopher's stone" has yet to be found. However, the insightful experiments of Bino *et al.* have opened up an avenue that might lead—hopefully in the near future—to the perfect alkyne metathesis catalyst. They have started to bridge the gap in mechanistic understanding that separates alkyne metathesis (*16*, *17*) with Schrock-type carbynes from that performed with in situ catalysts by finding a bona fide missing link.

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CHEMISTRY

Miniature Analytical Methods for Medical Diagnostics

David R. Walt

There has been a growing trend toward bringing medical diagnostic devices closer to the patient. Point-of-care devices for measuring electrolytes, cardiac markers, and several small molecules now reside in nursing stations, surgical suites, emergency rooms, and even at patient bedsides. Police carry breathalyzers for alcohol screening, while diabetics conduct routine glucose tests using credit card– or pen-sized devices. This Perspective describes the technologies underlying these advances, including microfluidics, arrays, sensors, and nanomaterials, and provides a glimpse of what lies in store in the future.

In addition to ions and small molecules, proteins, genes (DNA), and gene transcripts (mRNA) can now be measured routinely. In some cases, specific analytes or DNA sequences are diagnostic for a particular disease state, while in other cases, an overall profile correlates to a disease [for example, elevated LDL (low-density lipoprotein), cholesterol, and C-reactive protein are characteristic of cardiovascular disease (CVD)].

The discovery of clinically important analytes often involves large, sophisticated, and expensive analytical instrumentation such as mass spectrometers. The challenge then is to develop smaller, more focused instruments to monitor these analytes (see the figure). Advances in surface and materials chemistry, engineering, and the availability of new electronic components, such as light sources, memory chips, detectors, and integrated electronic components, have all contributed to advances in sensors. A focus has been on diabetes monitoring. One innovation is the GlucoWatch (*1*), an electrochemical glucose biosensor incorporated into a wristwatch-like device. When the patient wears the watch, electrical stimulation causes fluid to pass through the skin in a process called iontophoresis. A glucose sensor on the back of the watch analyzes the fluid, thereby providing a relatively continuous readout of glucose concentration that is reasonably accurate. A second new glucose biosensor [Therasense, Abbott (*2*)], requires a submicroliter ($<10^{-6}$ liter) blood sample that can be acquired virtually painlessly from many areas of the body, such as the forearm.

For acquiring parallel measurements of many analytes, arrays are unequaled (*3*–*7*). Most arrays contain hundreds to tens of thou-

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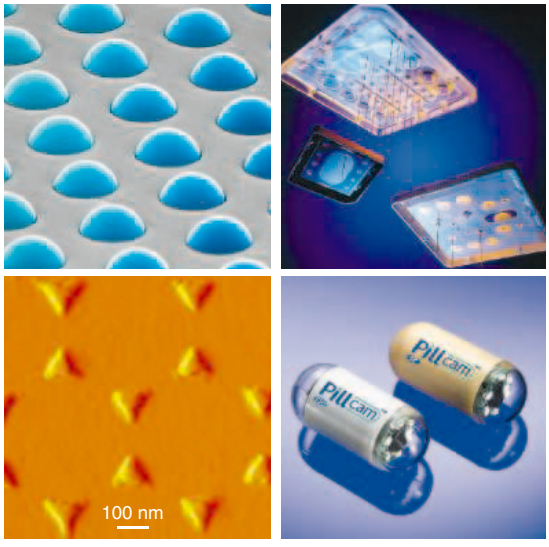
sands of micrometer (10^{-6} m)–sized features. Two strategies exist for performing array analysis—measuring multiple specific analytes simultaneously, or measuring patterns or profiles. Most arrays fall in the first category, with signals generated upon analyte binding to specific molecular receptors attached to each array element. Signals are typically either electronic, such as a change in current, or optical, such as a change in fluorescence. For example, DNA microarrays are now commercially available that contain all the known genes in the human genome (*8*–*11*). Although these arrays are primarily confined to research laboratories, the decreasing feature sizes of the array elements and the simplification in the readout instrumentation should enable the technology to become part of the clinical setting. As simpler, more focused arrays become available, such as DNA chips for cancer or infectious disease, the trend will be toward bringing these arrays even closer to the patient. An alternative approach to arrays is based on pattern recognition (*12*). Such sensor arrays are commonly referred to as "electronic noses," because they are based loosely on principles of the olfactory system. In this approach, a series of cross-reactive sensors generates a response pattern that is used to train a computational pattern recognition program—similar to the way in which mammals associate an odor with an odor source such as food, a mate, or a predator. For example, such an array can be trained to recognize the odor in breath associated with lung cancer (*13*, *14*). Challenges presented by these types of arrays are (i) the complex training protocols required for the system to learn to discriminate background from signal and (ii) producing reproducible arrays that do not require retraining when they replace the original array (*15*).

Sensors and arrays have been miniaturized, but there is also an effort to miniaturize and integrate sample preprocessing with analyte detection. Microfluidic or "lab on a chip" systems use small sample and reagent volumes coupled with integrated detection methods (*16*–*22*). These devices are fabricated in glass or plastic chips. In their most sophisticated manifestation, sample introduction, preprocessing (for example, cell lysis, dilution, and debris removal), reagent addition, and signal detection are all conducted on the chip. The entire chip, including integrated electronics or optics, can be the size of a typical microscope slide or may be in a compact disk format (*20*). Reagent and sample volumes can be on the order of picoliters (10^{-12} liters). Such systems have been crafted for DNA analysis, immunoassays, cell analysis, and enzyme-activity measurements (*23*, *24*). For now, most of these systems remain more complicated and bulkier than a simple integrated miniature device, because in many cases external optics, pumps, and detectors are required to control and read out signals from the chips. In addition, liquid reagent reservoirs must be incorporated into the systems so that they can be used for multiple samples. Reagent stability also remains a concern.



Detection on a small scale. (Clockwise from top left) Illumina bead array, Caliper microfluidic chip, GIVEN endoscopy capsule, and nanosilver pyramids for enhanced detection. [Figures courtesy of the listed companies and R VanDuyne, Northwestern University]

Related to microfluidic systems are microelectromechanical systems, referred to as MEMS devices. MEMS differ in that they are self-contained and do not require reagents. An example of such a system that has revolutionized internal medicine is the swallowed capsule technology for locating internal bleeding in the GI (gastrointestinal) tract [GIVEN Imaging (*25*)]. The patient swallows a capsule containing a light-emitting diode for illumination, a CMOS (complementary metal-oxide semiconductor) video camera and optics for taking images, a battery, and a transmitter. The device captures images of the entire GI tract and transmits them to an external receiver worn on the patient's belt. After traversing the GI tract, the capsule is excreted. The images are then compiled into a video that enables the physician to view the stomach and colon with high enough resolution to identify bleeding.

Nanotechnology is driving down the size of miniature diagnostic systems even further. Nanomaterials (materials with features of 100 nm or smaller) are being created with new properties and functions (*26*–*29*). In this size regime, material properties are different than at macroscopic scales, exhibiting phenomena such as electromagnetic field enhancement, quantum confinement, and signal amplification. These materials have desirable properties such as narrow emission band fluorescence, surface plasmon resonance, and conductivity, enabling new signaling and recognition phenomena for use in sensors. Nanomaterials can be targeted to tumor sites and used for imaging (*30*). Single carbon nanotubes or arrays of nanotubes can be used as sensors in which binding to the nanotube surface causes changes in conductivity, potential, or optical properties. The nanometer size enables measurements of extremely high density, high functionality, and high sensitivity (*31*, *32*). Although counterintuitive, it is easier to detect a small number of molecules in a small volume because the local concentration is higher than if one has the same number of molecules in a larger volume. On the other hand, when analyzing small volumes of low-concentration analytes, Poissonian sampling errors come into play. These errors arise because a small volume may contain too few molecules and not be representative of the true sample concentration.

As these technologies are brought closer to the patient, the nature of the samples will need to change. Blood, tissue from biopsies, urine, and stool have been the traditional clinical samples. A saliva or breath sample would be much more convenient and would encourage more frequent monitoring. For example, the U.S. Food and Drug Administration (FDA) recently approved OraQuick [OraSure Technologies (*33*)], an HIV test that uses a saliva sample. A collection pad on a stick is inserted between the lower cheek and gum and is left in place for 2 min to collect mucosal transudate, which contains more immunoglobulin G antibodies than saliva does. The sample is tested using an immunochromatographic assay, in which the sample mixes with reagents and binds to specific capture antibodies located on the test strip. If the test is positive, two lines (sample and control) appear, whereas a negative result provides only a single control line. The entire test takes 20 min.

As is common for medical and clinical diagnostic systems, several regulatory issues must be resolved before miniaturized diagnostic technologies become approved and accepted. In particular, the vast amount of data that can be collected on a single patient sample using arrays presents a challenge to regulatory agencies and clinicians alike. In addition, in an environment where many clinically relevant analytes are detected simultaneously, the present model of a physician ordering a specific test for a specific diagnosis that is reimbursed on a per test basis will have to change. Such large-scale tests may not be too

far away and offer the possibility of early diagnosis and treatment. Farther down the road may be personalized health care with diagnosis and disease-monitoring occurring in the home with easy-to-use miniature devices.

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RETROSPECTIVE: PHYSICS

Hans A. Bethe (1906–2005)

Freeman Dyson

Theoretical physicists come in two varieties: deep thinkers and problem solvers. Hans Bethe was the supreme problem solver of the past century. He was not a deep thinker like Heisenberg and Dirac, who laid the foundations of modern physics in the 1920s. But he took their theories and made them into practical tools for understanding the behavior of atoms, stars, and everything in between. He might have described his contribution to science with the words that Isaac Newton used to introduce the third and final volume of his *Principia Mathematica*: "It remains that, from the same principles, I now demonstrate the frame of the system of the world."

Bethe demonstrated how quantum theory can explain phenomena as diverse as the supernova explosions of stars in the sky, the multiplication of cosmic-ray showers in the atmosphere, and the fine structure of the energy levels in the microwave spectrum of a hydrogen atom. He wrote a comprehensive review of nuclear physics and demonstrated how nuclear reactions keep the Sun and the stars shining. In 1967, he was awarded the Nobel Prize for physics for his work on the Sun and stars. But his studies of the energy levels of hydrogen were at least as important and even more fundamental, demonstrating the effects of quantum fluctuations of the electromagnetic field on the dynamics of atoms. His 1947 paper on the hydrogen atom (*1*) was only three pages long, but it set the style for theoretical calculations in particle physics for the next 50 years.

On Bethe's desk at Cornell University, where he lived and taught for almost 70 years, there was always a pad of paper that he used for calculations. His door was usually open; students and colleagues came in constantly to discuss a wide variety of problems. Bethe would instantly switch his attention from his own problem to theirs. As soon as they left the room, he would instantly switch his attention back and continue his calculation where he had left off.

He continued to pour out a stream of research papers while carrying a full load of teaching and administrative duties and supervising an army of graduate students. When I was one of his graduate students, he came every day to eat lunch with us at the student cafeteria, sharing our problems and telling stories of his adventures in Germany and in Los Alamos. We learned even more at the lunches than we did at his lectures. Everyone called him Hans. He told us that one of the best things about moving from Germany to America was that nobody in America called him "Herr Professor."

Bethe remained active as a physicist, doing calculations and publishing papers, well into his nineties. From the age of 70 to the age of 95, he enjoyed a fruitful collaboration with Gerald Brown, working out theories of supernova explosions and gamma-ray bursts. Brown has published a delightful account of the collaboration, with the title "Fly with Eagles" (*2*). Brown says, "I had to wait until he was more than 70 years old in order to have any chance of keeping up with him. He worked like a bull-dozer, heading directly for the light at the end of the tunnel." The last time I talked with Bethe, he said, "It is a shame. Now I am 98 and I cannot be as active as I was when I was 90."

Bethe carried in his head all the numbers that play an important role in physics or in engineering. Given any question, he could estimate a numerical answer with lightning speed. His estimates were amazingly accurate. He put this skill to good use when he helped Robert Wilson to design a succession of particle accelerators at Cornell. The same skill made him an ideal leader for the theoretical division at Los Alamos, designing the first atomic bomb during World War II and helping to design the first hydrogen bomb in 1952.

For the 60 years that he lived after 1945, he worked hard to educate the public about the facts of nuclear weaponry and the impossibility of winning a nuclear war. He was actively engaged in fighting for arms-control treaties and against escalations of the arms race. At the age of 90, he wrote in a letter to President Clinton: "The time has come for our nation to declare that it is not working, in any way, to develop further weapons of mass destruction of any kind. …You might consider making a suitable pronouncement along these lines, to discipline the bureaucracy, and to reassure the world that America is vigilant in its desire to ensure that new kinds of nuclear weapons are not created." Now that he is dead, it is up to us to continue the good fight that he fought for nuclear sanity, moderation, and common sense.

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Homeward Sound

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Most reef populations are replenished with recruits that settle out from an initially pelagic existence. The larvae of nearly all coral reef fish develop at sea for weeks to months before settling back to reefs as juveniles. Although larvae have the potential to disperse great distances, recent studies show a substantial portion recruit back to their natal reefs (*1*, *2*). Larvae are not passively dispersed but develop a high level of swimming competence (*3*). How they use these capabilities to influence their dispersal is an open question. We show here that recruits respond actively to reef sounds, potentially providing a valuable management tool for the future.

Since the discovery that reef fish larvae are accomplished swimmers, focus has shifted to identifying cues that may influence their orientation. Sound has emerged as a leading candidate, because it travels in water irrespective of current flow with little attenuation and because fish and invertebrates create a clamour that can be heard for many kilometers around (*4*). We have previously shown the attraction of settlement-stage reef fishes from many families to reef noise, using light traps and prerecorded sound (*5*). Here we provide direct evidence that sound enhances settlement of fish onto patch reefs.

We used two experiments to study settlement behavior in the presence of recorded reef sounds (*6*). In November 2003, we built 24 patch reefs from dead coral rubble on sand flats in 3- to 6-m-deep water at Lizard Island on the Great Barrier Reef (fig. S1). For six nights, we deployed submersible speakers broadcasting reef noise (at 156 dB relative to 1 μPa at 1 m, mostly the sound of snapping shrimp and fish calls) on 12 of these patch reefs, alternating the location of the speakers each night. Most settlement occurs at night, so recruiting fish were collected from the patch reefs early the following mornings. Of the 868 recruits we collected, most were apogonids (or cardinalfish, 80%) or pomacentrids (or damselfish, 15%). These two families are key members of coral reef fish assemblages around the world: The apogonids contribute up to one quarter of all individuals on reefs and the pomacentrids up to half of the total fish biomass (*7*). Analyses showed no site or date effects in our data, but both families settled in greater numbers on noisy patch reefs than on silent reefs (Fig. 1A). A preference for noisy patch reefs was also seen in less common fishes, with marginally more taxa (excluding apogonids and pomacentrids) on patch reefs with broadcast noise than on reefs without (Fig. 1B).

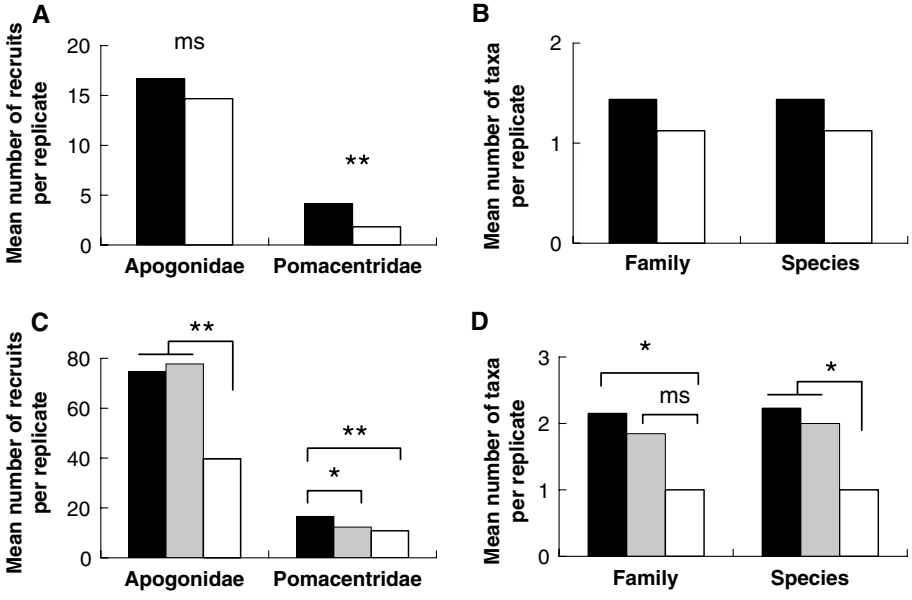

Fig. 1. Comparison of catches from patch reefs with different sound treatments (tables S1 to S3). (**A** and **B**) Reefs broadcasting reef noise (black) or silent reefs (white). (**C** and **D**) Reefs with high-frequency (black) or low-frequency (gray) reef noise or silent reefs (white). Statistical results are for (A) Chi-squared analyses, (B) Wilcoxon's matched pairs test, (C) pairwise Chi-squared analyses with Bonferroni corrections, and (D) pairwise Wilcoxon's matched pairs test with Bonferroni corrections (ms, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$). All apogonids and pomacentrids were excluded from the analyses in (B) and (D).

In December 2003, the experimental field site was used to compare the settlement of fishes to patch reefs where we broadcast primarily the high frequencies of reef noise (80% > 570 Hz, predominantly shrimp) or low frequencies of reef noise (80% < 570 Hz, predominantly fish) with settlement to silent reefs. This time, nearly four times as many recruits arrived (3111 fish), but the taxonomic composition was similar. Apogonids settled on high- and low-frequency patch reefs in equivalent numbers, but pomacentrids were preferentially attracted to reefs with high-frequency noise (Fig. 1C). Again, reefs without sound received less settlement from rarer taxa than reefs with broadcast sound (Fig. 1D).

This study provides direct field evidence that settling reef fishes use sounds to orientate toward and select reefs. Furthermore, there is an indication that some fish groups may be selectively using specific components of the reef sound to guide their settlement behavior. The important use of sound at this critical life history phase raises the possibility of potential adverse effects of increasing anthropogenic noise pollution (e.g., shipping and drilling), but it may also lead to the development of new tools for fisheries managers for restocking fisheries or newly established marine reserves.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5719/221/DC1
Materials and Methods
Fig. S1
Tables S1 to S3

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Murine Reference Reagents Available:
IFN-α; IFN-β; IFN-γ; IL-3; GM-CSF.

To obtain Reference Reagents outside the United States,
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For questions contact:
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Supra-Canonical $^{26}Al/^{27}Al$ and the Residence Time of CAIs in the Solar Protoplanetary Disk

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The canonical initial $^{26}Al/^{27}Al$ ratio of 4.5×10^{-5} has been a fiducial marker for the beginning of the solar system. Laser ablation and whole-rock multiple-collector inductively coupled plasma-source mass spectrometry magnesium isotope analyses of calcium- and aluminum-rich inclusions (CAIs) from CV3 meteorites demonstrate that some CAIs had initial $^{26}Al/^{27}Al$ values at least 25% greater than canonical and that the canonical initial $^{26}Al/^{27}Al$ cannot mark the beginning of solar system formation. Using rates of Mg diffusion in minerals, we find that the canonical initial $^{26}Al/^{27}Al$ is instead the culmination of thousands of brief high-temperature events incurred by CAIs during a 10^5-year residence time in the solar protoplanetary disk.

The short-lived radionuclide ^{26}Al [mean life = 1.05 million years (My)] was an important source of heat in the early solar system (*1*). It is also a high-resolution chronometer for early solar system evolution (*2*). Its former presence in the solar system is evidenced by excesses in its decay product, $^{26}Mg^*$, correlated with Al/Mg in the constituents of meteorites (*3*). The largest excesses occur in the CAIs found in many chondrites. The CAIs are the oldest known solid objects that formed in the solar system and have absolute ^{207}Pb-^{206}Pb ages of 4567.2 ± 0.6 My (*4*). Here, we present new ultraviolet (UV) laser ablation and acid digestion multiple-collector inductively coupled plasma-source mass spectrometry (MC-ICPMS) analyses of CAIs showing that there was more ^{26}Al in the early solar system than previously thought and that the canonical $^{26}Al/^{27}Al$ is a reflection of the residence time of CAIs in the protoplanetary disk.

The use of ^{26}Al as a chronometer relies on variations in the initial $^{26}Al/^{27}Al$ ratio [$(^{26}Al/^{27}Al)_o$] in objects formed within several mean lives of ^{26}Al decay. Values for $(^{26}Al/^{27}Al)_o$ are defined by isochrons comprising linear correlations between $^{26}Mg^*/^{24}Mg$ and $^{27}Al/^{24}Mg$. The slopes of these correlations are numerically equivalent to $(^{26}Al/^{27}Al)_o$ because all of the ^{26}Al decayed away billions of years ago. Age differences are reflected in differences in $(^{26}Al/^{27}Al)_o$ if $^{26}Al/^{27}Al$ was uniform in the early solar system.

A central assumption is that the canonical $(^{26}Al/^{27}Al)_o$ value equates with an absolute age of about 4567 My and represents the initial abundance of ^{26}Al for the solar system as a whole. Most data have come from measurements of Al-rich minerals, especially anorthite, a Ca-rich feldspar mineral, because high $^{27}Al/^{24}Mg$ affords high-precision estimates of $^{26}Mg^*$. The canonical $(^{26}Al/^{27}Al)_o$ for the solar system based on these data is 4.5×10^{-5} (*5*, *6*). It has been thought that all reliable measurements of $(^{26}Al/^{27}Al)_o$ are 5×10^{-5} or less (*5*); past claims of $(^{26}Al/^{27}Al)_o$ values greater than 5×10^{-5} have been dismissed as spurious as a result of analytical errors (*5*).

Some, though not all (*7*), MC-ICPMS measurements of $^{26}Mg^*$ and Al/Mg in low-Al CAI minerals have suggested that the initial $(^{26}Al/^{27}Al)_o$ of the solar system may have been higher than the canonical value (*8*–*10*). These early data were viewed as too few to warrant revision of the canonical $(^{26}Al/^{27}Al)_o$. We present new laser-ablation MC-ICPMS Mg isotope data for six igneous CAIs from the Allende (M5, USNM 3576-1), Efremovka (E44), Grosnaja (63624-1), and Leoville (144A, MRS3) CV3 meteorites and two "fluffy" type A CAIs from the Vigarano CV3 meteorite (Vigarano 10 and Vigarano 9) (*11*). In addition, we present analyses of dissolved fragments representing substantial parts of three of the objects (Allende 3576-1, Grosnaja 63624-1, and Leoville 144A) to provide estimates of their bulk Mg isotopic compositions.

Supra-canonical $^{26}Al/^{27}Al$. Of the 284 laser-ablation analyses of the eight CAIs (table S1), most (79%) lie above the canonical line corresponding to $(^{26}Al/^{27}Al)_o = 4.5 \times 10^{-5}$ (Fig. 1). The $\delta^{26}Mg^*$ [the per mil (‰) excess in $^{26}Mg/^{24}Mg$ due to the presence of $^{26}Mg^*$] and $^{27}Al/^{24}Mg$ values are correlated, and the data for six of the eight objects are consistent with a zero intercept; the laser ablation data include isochrons corresponding to $(^{26}Al/^{27}Al)_o$ values greater than canonical. The three bulk (whole-rock) CAI values for Allende 3576-1, Grosnaja 63624-1, and Leoville 144A agree with the laser-ablation data for these objects; whole-rock and laser-ablation $\delta^{25}Mg'$ and $\delta^{26}Mg'$ values ($^{25}Mg/^{24}Mg$ and $^{26}Mg/^{24}Mg$ ex-

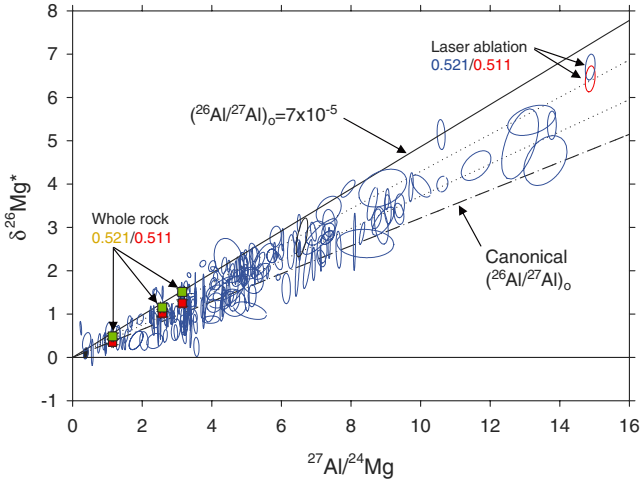

Fig. 1. Compilation of 203 analyses obtained by laser-ablation MC-ICPMS representing seven of the eight CAIs in this study (another 81 analyses of Grosnaja 63624-1 are omitted for clarity). Each datum is shown as a 1σ error ellipse. 79% of analyses are above the canonical $(^{26}Al/^{27}Al)_o$ line. $\delta^{26}Mg^*$ correlates with $^{27}Al/^{24}Mg$, and many data (e.g., 144A, Fig. 2) are consistent with a zero intercept, all of which are requisites for interpreting the results as indicative of decay of ^{26}Al with $(^{26}Al/^{27}Al)_o > 4.5 \times 10^{-5}$. Also shown are the three whole-rock CAI values represented by green squares (error bars are smaller than symbols). Red squares and the single red ellipse show the effects of recalculating the whole-rock data and the laser-ablation analyses, respectively, with a mass fractionation relationship between $\delta^{25}Mg'$ and $\delta^{26}Mg'$ of 0.511 rather than 0.521. The $(^{26}Al/^{27}Al)_o$ values of the lines in descending order from top are 7×10^{-5} (solid), 6×10^{-5} (dot), 5.2×10^{-5} (dot), 4.5×10^{-5} (canonical, dash-dot), and 0.0 (solid).

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pressed as per mil differences from a standard) of the objects at comparable $^{27}Al/^{24}Mg$ are consistent (table S1). Weighted linear regression (*12*) of the whole-rock data gives a model $(^{26}Al/^{27}Al)_o$ isochron of 7.0 (±1.32 2σ) × 10^{-5}, a $\delta^{26}Mg^*$ intercept of −0.1 (±0.2 2σ), and an MSWD (mean square weighted deviation) of 0.37. Regression of the whole-rock data with a 0.511 rather than a 0.521 mass-dependent isotope fractionation relationship between $\delta^{26}Mg'$ and $\delta^{25}Mg'$ gives 6.3 (±1.3 2σ) × 10^{-5}, −0.2 (±0.2 2σ), and 0.08 for $(^{26}Al/^{27}Al)_o$, $\delta^{26}Mg^*$ intercept, and MSWD, respectively.

Data for the two most thoroughly studied CAIs, 144A, a compact type A from Leoville, and E44, a type B1 from Efremovka, are used here to illustrate the evidence for high $(^{26}Al/^{27}Al)_o$ values as defined by intra-CAI relationships obtained by UV laser-ablation MC-ICPMS. (For comparison, Leoville MRS3 is most like 144A in its isotope systematics, whereas Grosnaja 63624-1 is similar to E44). Weighted linear regression of the complete data set for 144A defines a $(^{26}Al/^{27}Al)_o$ value of 5.9 (±0.3 2σ) × 10^{-5}, with an intercept of 0.0 (±0.07 2σ) and an MSWD of 3.3 (Fig. 2 and table S1). An MSWD >1 indicates real variability in the data beyond analytical uncertainties. The isochron is dominated by melilites (with minor inclusions of spinel), because the melilite $Al_2Mg_{-1}Si_{-1}$ crystallographic substitution produces the greatest spread in Al/Mg in the CAI. Regression of the melilite data gives a line similar to the line defined by the combined data (and with the same MSWD). The data span up to the $(^{26}Al/^{27}Al)_o$ = 7 × 10^{-5} line defined by some bulk CAIs in this study and in (*10*). We conclude that inclusion 144A shows evidence for $(^{26}Al/^{27}Al)_o$ of at least 6 × 10^{-5} and probably higher.

Data for Efremovka E44 define a canonical value for $(^{26}Al/^{27}Al)_o$ (Fig. 3 and table S1). Results from this inclusion are relevant to the evidence for supra-canonical $(^{26}Al/^{27}Al)_o$ for two reasons: (i) this result (and similar results for Grosnaja 63624-1) shows that MC-ICPMS measurements reproduce the canonical value for $(^{26}Al/^{27}Al)_o$ that is so common among CAI anorthites; and (ii) the E44 data reveal the reason for the prevalence of the canonical $(^{26}Al/^{27}Al)_o$ values among CAIs and among anorthites in particular.

Results for all analyses of E44 define a line with a slope corresponding to $(^{26}Al/^{27}Al)_o$ = 4.8 (±0.3 2σ) × 10^{-5} and an intercept of 0.3 (±0.1 2σ) ‰ (Fig. 3). The MSWD for the E44 data combined is 2.3. Melilites from E44 taken by themselves define a different line corresponding to $(^{26}Al/^{27}Al)_o$ = 4.3 (±0.5 2σ) × 10^{-5}, with an intercept of 0.7 (±0.2 2σ) ‰ and an MSWD of 1.0 (Fig. 3). We interpret the unit MSWD of the melilites compared with the higher value for the combined data as an indication that melilites represent a single population (an MSWD of 1 indicates a single population), whereas the pooled data represent several populations marked by isotopic discordance.

The nonzero intercept for E44 melilites (Fig. 3) is a manifestation of a nonzero initial $\delta^{26}Mg^*$ and indicates exchange with a reservoir with higher $^{26}Mg^*/^{24}Mg$ and Al/Mg ratios. The only phase with higher Al/Mg and $^{26}Mg^*/^{24}Mg$ than melilite in E44 is anorthite, and addition of anorthite to the melilite regression leaves the latter unchanged; melilite and anorthite are on the same line. Similar relations involving melilite have been attributed to subsolidus closed-system isotopic exchange between anorthite and melilite (*13*). Closed-system exchange of Mg isotopes between these phases to the exclusion of others is expected because both minerals are characterized by relatively high rates of Mg self-diffusion (*14*, *15*).

Mg isotope exchange between melilite and anorthite has been seen in other CAIs, but the data for E44 are revealing because they show signs of exchange despite exhibiting a canonical $(^{26}Al/^{27}Al)_o$. Because the line defined by the melilite and anorthite data in E44 is probably the result of exchange of Mg isotopes between these phases, it follows that the anorthite complement of $^{26}Mg^*$ corresponding to the canonical $(^{26}Al/^{27}Al)_o$ is not representative of the initial value for the solar system. For the melilite $\delta^{26}Mg^*$ to define a nonzero intercept of 0.7 ‰ (Fig. 3), the anorthite with which it exchanged would have to have experienced a decrease in $\delta^{26}Mg^*$ of ~25 ‰ at some point during the history of the CAI. The alternative is that the melilite alone exchanged Mg isotopes with an outside reservoir enriched in $\delta^{26}Mg^*$ relative to its Al/Mg. Such an open-system exchange cannot be ruled out a priori, but it is difficult to explain why melilite would be susceptible to exchange, whereas anorthite was not given their comparably high Mg diffusivities (*14*, *15*). Open-system exchange also offers no explanation for the coincidence of the melilite and anorthite apparent isochrons.

Our data are consistent with a $(^{26}Al/^{27}Al)_o$ value of at least 6 × 10^{-5} and could be reconciled with the higher value of 7 × 10^{-5} recorded by some whole-rock CAIs (Fig. 1) if the Mg isotopes were disturbed as indicated by the MSWD >1 for Leoville 144A. In what follows, we use the conservative value of 6 × 10^{-5} defined by the best-fit isochron for 144A (Fig. 2).

Significance of supra-canonical $^{26}Al/^{27}Al$. Differences between supra-canonical and canonical $(^{26}Al/^{27}Al)_o$ values could be due to differences in time prescribed by the decay equation $(^{26}Al/^{27}Al)_t = (^{26}Al/^{27}Al)_o \exp(-\lambda \Delta t)$, where Δt is in years and $\lambda = 9.52 \times 10^{-7}$ year^{-1}, or they could result from differences in the Al isotope compositions of the reservoirs from which different CAIs formed. The nonzero initial $\delta^{26}Mg^*$ value for a CAI with canonical $(^{26}Al/^{27}Al)_o$ and the prevalence of the canonical $(^{26}Al/^{27}Al)_o$ value are most easily understood if the spread in $(^{26}Al/^{27}Al)_o$ has chronological importance. The indicated time interval Δt before final closure of the ^{26}Al-$^{26}Mg^*$ system based on the above expression, where $(^{26}Al/^{27}Al)_{(t)} = 4.5 \times 10^{-5}$ and $(^{26}Al/^{27}Al)_o$ = 6.0 × 10^{-5}, is 300,000 years. The widespread nature of the 4.5 × 10^{-5} value in feldspars (and in some other minerals) among CAIs supports the interpretation that these minerals record ^{26}Al-$^{26}Mg^*$ closure with a common solar-system-wide $(^{26}Al/^{27}Al)_o$. This closure must have followed nearly complete early resetting of the ^{26}Al-$^{26}Mg^*$ system. The alternative of different degrees of partial resetting for different objects would not result in a well-defined canonical value once ^{26}Al had decayed entirely to $^{26}Mg^*$.

The nonzero initial $\delta^{26}Mg^*$ of 0.7 (±0.2) ‰ for E44 melilite is consistent with exchange of Mg isotopes between melilite and feldspar for 300,000 (±100,000) years after initial growth

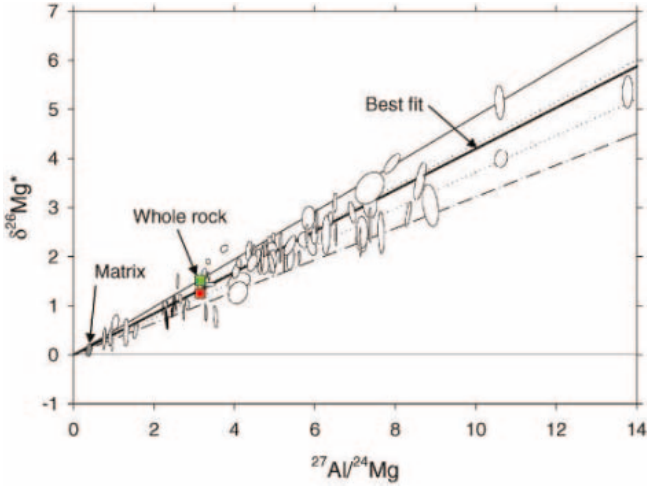

Fig. 2. Plot of $\delta^{26}Mg^*$ versus $^{27}Al/^{24}Mg$ values for CAI 144A (compact type A) from the Leoville CV3 meteorite obtained by laser-ablation MC-ICPMS. Each datum is shown as a 1σ error ellipse. Most data are above the canonical line (dash-dot line), and many are on or just below the 7 × 10^{-5} line (upper solid line) defined by some bulk CAI data. The data show a strong correlation with a best-fit slope corresponding to $(^{26}Al/^{27}Al)_o$ = 5.9 × 10^{-5} (heavy black line). Also shown is the whole-rock datum for 144A calculated for two different reference fractionation lines (green square and red square as in Fig. 1). The $(^{26}Al/^{27}Al)_o$ values of the reference lines are the same as in Fig. 1.

(Fig. 3), which supports the Δt of 300,000 years obtained from the spread in $(^{26}Al/^{27}Al)_o$ values. After 300,000 years of decay of ^{26}Al with $(^{26}Al/^{27}Al)_o = 6 \times 10^{-5}$, the $\delta^{26}Mg^*$ for the bulk $^{27}Al/^{24}Mg$ (volume-weighted average) composition of melilite and anorthite in E44 (the bulk $^{27}Al/^{24}Mg$ is ~6.5) would be 0.7 ‰. The values for 200,000 years and 400,000 years are 0.5 and 0.9 ‰, respectively, spanning the 0.2 ‰ uncertainty in the intercept. Because 0.7 ‰ is the value for the bulk composition consisting of melilite and anorthite in E44, complete isotopic equilibration between these minerals after 300,000 years would have resulted in both having an initial $\delta^{26}Mg^*$ of 0.7 ‰ (Fig. 3). Subsequent decay of ^{26}Al produces the observed melilite and anorthite data with a canonical $(^{26}Al/^{27}Al)_o$, reflecting the $^{26}Al/^{27}Al$ of 4.5×10^{-5} at Δt = 300,000 years, but with an initial $\delta^{26}Mg^*$ of 0.7 ‰ rather than 0 (Fig. 3) (*11*).

Thermal resetting of $^{26}Al/^{27}Al$ in CAIs. Our data constrain the thermal history of CAIs in the solar nebula. The constraints come from the rate of diffusion of Mg isotopes in melilite and anorthite (*14*, *15*) and the result that these minerals continued to exchange Mg isotopes until ~300,000 years after initial CAI growth (*11*). We constructed a model for diffusive homogenization of $^{26}Mg^*/^{24}Mg$ between anorthite and melilite for various volume fractions of the two minerals to assess the combinations of time and temperature required for resetting during a 300,000-year interval after initial CAI formation. In our calculations, we considered separately the effects of Mg isotope diffusion and $^{26}Mg^*$ accumulation due to ^{26}Al decay. Resetting after only 300,000 years (a time less than the mean life of ^{26}Al) ensures that these two processes occurred simultaneously. In this case, growth of $^{26}Mg^*$ in feldspar and melilite could have been punctuated by many episodes of diffusion during heating until final closure of the system after 300,000 years. The overall effect is the same as that portrayed in our calculation. Our model was calculated (*11*) for a concentration of $^{26}Mg^*$ in anorthite and melilite appropriate for 300,000 years of decay of ^{26}Al, with an initial $^{26}Al/^{27}Al$ of 6.0×10^{-5} and $^{27}Al/^{24}Mg$ values of 256 and 2.56 representing anorthite and melilite, respectively (yielding initial $\delta^{26}Mg^*$ values of 27.3 ‰ for anorthite and 0.3 ‰ for melilite before diffusive isotope exchange). Inclusion E44 has a radius of ~5 mm, so we used this size for the model.

The results are made universally applicable to the problem of melilite-anorthite Mg isotope exchange by presenting them in terms of the diffusion-reaction progress variable ξ (*16*). This progress variable is defined as:

$$\xi = \int_0^t \frac{D(T)}{z^2}\,dt \qquad (1)$$

where $D(T)$ is the temperature-dependent diffusion coefficient for Mg isotope exchange, z is the dimension of the diffusion medium, and t is time. Values for ξ show the combinations of the effective temperature (time-integrated temperature) and the time necessary to effect a specified amount of diffusive $^{26}Mg^*/^{24}Mg$ exchange between anorthite and melilite (Fig. 4).

The value of ξ required for complete homogenization of $\delta^{26}Mg^*$ between anorthite and melilite by Mg diffusion, where the difference between $\delta^{26}Mg^*$ in anorthite and melilite becomes 0, is 0.2 for the anorthite/(anorthite + melilite) = 0.25 volume fraction applicable to E44 (Fig. 4A). The attendant increase in melilite $\delta^{26}Mg^*$ of several tenths of a per mil due to the resetting is consistent with the observed excess in $\delta^{26}Mg^*$ in melilite at the model $^{27}Al/^{24}Mg$ (Fig. 3).

The resetting of the Mg isotopic system in melilite and anorthite requires 300 years at near-solidus temperatures of 1600 K (Fig. 4C). We adopt 1600 K as an absolute maximum subsolidus temperature for anorthite + melilite in CAIs but one that allows for partial melting of components near the eutectic (*17*, *18*). At 900 K, resetting takes 10^9 years. If conditions were suitable for resetting ^{26}Al-$^{26}Mg^*$ in E44 anorthite, they were more than sufficient to reset feldspars in most other CAIs (*19*) (Fig. 4) and probably in smaller Al-rich and Mg-poor grains of other minerals. CAIs with fewer, smaller anorthite grains would require lower ξ and, therefore, less time and temperature; other high-Al minerals of small size, perhaps with lower Mg diffusivities (e.g., hibonite grains), could also be reset under these conditions because the critical factor is $\xi\ z^2$ rather than $D(T)$ alone. For this reason the ξ for E44, an especially anorthite-rich CAI with unusually large grain size, represents a maximum for CAIs.

High temperatures that caused Mg diffusion like that evidenced in E44 must have occurred in the solar nebula. This is because the time scale for resetting the ^{26}Al-$^{26}Mg^*$ system at peak temperatures appropriate for asteroid-like parent bodies (~900 K) is 10^9 years and is inconsistent with parent-body thermal models, meteorite isotopic data, and geological evidence (*20*, *21*). This conclusion in no way detracts from the importance of later parent-body processes such as metamorphism and aqueous alteration that undoubtedly lead to further ^{26}Al-$^{26}Mg^*$ discordance in some CAIs.

Residence time of CAIs in the protoplanetary disk. The Δt of ~300,000 years indicated by our $(^{26}Al/^{27}Al)_o$ data is a reasonable estimate of the residence time τ for CAIs in the protoplanetary accretion disk of dust and gas that surrounded the sun. This is because Δt should also represent the time interval between initial CAI formation and the cessation of thermal processing and resetting of the ^{26}Al-$^{26}Mg^*$ chronometer; the residence time τ should be about equal to Δt. The resetting marked by Δt can be explained as the

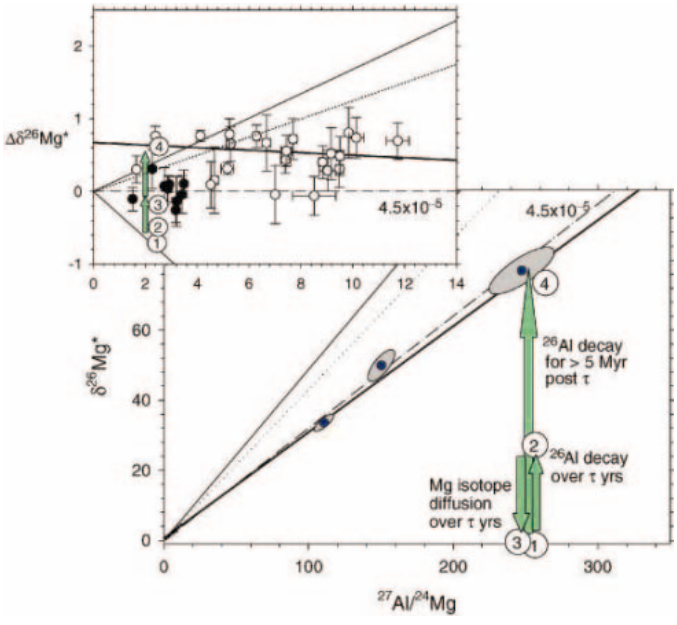

Fig. 3. Laser-ablation MC-ICPMS analyses of Efremovka CAI E44. Feldspars are shown as gray 1σ error ellipses in the lower panel, melilite and Al-Ti diopside as open and solid circles in the upper panel, respectively. The lower panel shows that a linear best fit for melilites and feldspars in E44 (heavy black line) is close to the canonical line (dash-dot line). The ordinate in the upper panel, $\Delta\delta^{26}Mg^* = \delta^{26}Mg^* - 0.32\ (^{27}Al/^{24}Mg)$, is the difference between $\delta^{26}Mg^*$ and the corresponding value for the canonical $(^{26}Al/^{27}Al)_o$ of 4.5×10^{-5} at the same $^{27}Al/^{24}Mg$. Error bars are 1σ. In this plot, the canonical evolution line is horizontal, and the absence of $^{26}Mg^*$ is shown by the lowermost negatively sloping solid line. The upper plot shows that melilites from E44 are above the canonical line and define an evolution line with a nonzero intercept of 0.7 ‰. All data for minerals other than feldspar for E44 appear in the lower left corner of the lower plot for reference. Also shown as numbered circles and arrows in the lower and upper panels is the evolution of feldspar and melilite, respectively. The minerals evolve by ^{26}Al decay and Mg isotope diffusive exchange in the first 300,000 years (the approximate residence time τ in the nebula) followed by uninterrupted ^{26}Al decay to reach their final compositions. The 7×10^{-5} and 6×10^{-5} reference lines from Fig. 1 are shown.

culmination of exposure to thousands of short-lived heating events for 10^5 years in the nebula.

The time that any solid particle of millimeter size spent en route to the sun from several astronomical units (AU) in the surrounding protoplanetary disk was likely to have been about 10^4 years (*22*). Because the radial velocity of the grains is proportional to circumstellar radius R, most of this time was spent inside of 3 AU in the protoplanetary disk (*22*), where high temperatures were most likely. One site where high temperatures prevailed was near the growing sun ($R \sim 0.06$ AU) (*23*). Temperatures approaching and/or exceeding the melting point of CAIs have been postulated for the "reconnection ring" region of the gap between the accretion disk and the nascent sun (*23*, *24*). Here, proto-CAIs were exposed to flares and ambient temperatures that heated them to temperatures of about ≤1700 K (*24*). The residence time of a CAI in this region is believed to have been approximately 10 to 20 years (*24*). With a residence time in the ring of 20 years and a total high-temperature exposure time of ~300 years [from the diffusion progress ξ at 1600 K (Fig. 4)], CAIs like E44 would have had to enter the zone of heating about 15 times during their lifetimes in the nebula to reset the ^{26}Al-^{26}Mg* melilite-anorthite system. The multiple trips to the hot zone can be explained by entrainment of the CAIs in magneto-centrifugally driven x-winds emanating from the inner edge of the disk. In this way, the CAIs are launched back out to the more distal regions of the disk. The transport of material back to the disk by this process is considerably faster than inward drift rates through the disk (*24*). Although unlikely, if each trip from $R \geq 3$ AU to the reconnection ring took about 3×10^4 years (*22*), the total time required is about 4.5×10^5 years [15 trips × (3×10^4) years per trip]. The combination of ξ for Mg isotope diffusion and estimated transport times therefore indicates a nebular τ of 10^5 years for CAIs.

Another means of imparting high temperatures to CAIs is by passage through shock waves in the disk. Shock heating has been used to explain the cooling histories of chondrules, for example. Models for the process of shock heating in the nebula suggest that millimeter objects passing through the high-density waves experience temperatures of 1400 K to 2200 K for up to one day (*25*). Such events would have disturbed the ^{26}Al-^{26}Mg* system in CAIs after their formation.

Wood (*26*) proposed that spiral density waves in the nebula could be the shock waves responsible for heating rock materials. He described a circumstance in which two waves symmetrically distributed in the nebula travel with orbital periods on the order of 900 years, independent of circumstellar distance R. For material in quasi-Keplerian orbit in the inner solar nebula, these waves would have behaved as if they were effectively stationary. In this situation, the total time required to achieve resetting of the ^{26}Al-^{26}Mg* system in anorthite and melilite (300 years at 1600 K for ξ = 0.2) by passage of CAIs through the shocks is

$$\tau = (1/R)\int_0^R \Omega^{-1} d\mathrm{R}(N/2) \qquad (2)$$

where Ω is the Keplerian angular velocity $[R/(1\ \mathrm{AU})]^{-3/2}$ and N is the number of 1-day shock episodes required to achieve the requisite value for ξ. In this case, N must add up to 300 years of heating, which requires that $N = 300 \times 365 = 109{,}500$ 1-day shocks. The τ obtained from this scenario for a CAI drifting inward from about 3 AU is 2.3×10^5 years. The precise value for τ is sensitive to the exact time-integrated temperature of processing, but for reasonable effective shock temperatures of ≥ ~1500 K, the indicated nebular residence time τ is on the order of 10^5 years (the value for τ using 1500 K is 8×10^5 years). Melting of a CAI at higher temperatures would also reset the ^{26}Al-^{26}Mg* chronometer by rapid diffusion but can not explain the data for E44.

In both scenarios for high-temperature processing, we obtain a τ of 10^5 years. The derived τ explains the resetting of $(^{26}\mathrm{Al}/^{27}\mathrm{Al})_o$ in some CAIs from $\geq 6 \times 10^{-5}$ to 4.5×10^{-5} and the nonzero initial δ^{26}Mg* in E44 and in many other CAIs (*13*, *27*). Combining this result with the 10^4 years required for inward drift from 3 AU or beyond (*22*) suggests that CAIs like those examined here made several passes through the inner protoplanetary disk.

The meaning of the canonical $(^{26}\mathrm{Al}/^{27}\mathrm{Al})_o$ as a reflection of τ should apply to most CAIs, because conditions suitable for resetting ^{26}Al-^{26}Mg* in E44 anorthite in the first 10^5 years are more than sufficient to reset most feldspars and probably other, smaller Al-rich Mg-poor minerals typical of CAIs. Accordingly, Mg isotope resetting in CAIs with similar τ should record the same "canonical" $(^{26}\mathrm{Al}/^{27}\mathrm{Al})_o$, because it arises from uninterrupted ^{26}Al decay for $t > \tau$. Where $(^{26}\mathrm{Al}/^{27}\mathrm{Al})_o$ is less than canonical, Al isotope heterogeneity

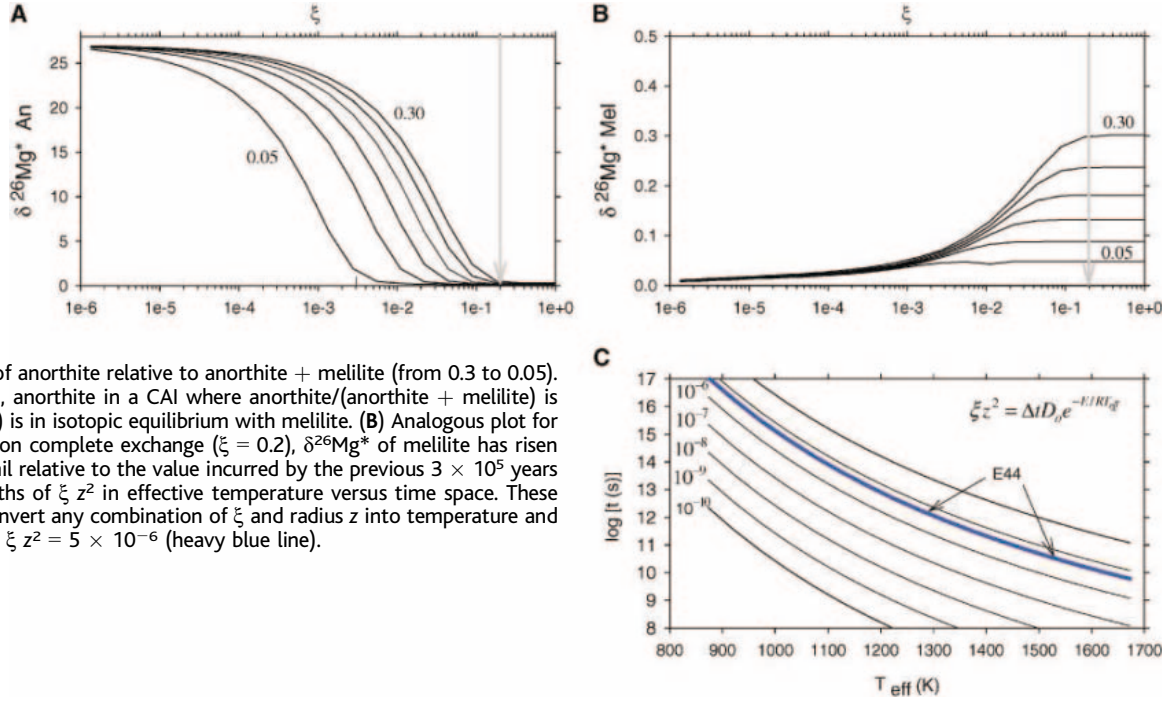

Fig. 4. Results of diffusion calculations expressed in terms of the diffusion-reaction progress variable ξ for a 10-mm diameter CAI (i.e., the size of E44). The initial condition corresponds to 3×10^5 years of ^{26}Al decay. (**A**) The reduction in δ^{26}Mg* in anorthite as measured relative to the initial melilite value. Isopleths are for different volume fractions of anorthite relative to anorthite + melilite (from 0.3 to 0.05). At ξ = 0.2 (gray arrows), anorthite in a CAI where anorthite/(anorthite + melilite) is 0.25 by volume (i.e., E44) is in isotopic equilibrium with melilite. (**B**) Analogous plot for melilite showing that upon complete exchange (ξ = 0.2), δ^{26}Mg* of melilite has risen several tenths of a per mil relative to the value incurred by the previous 3×10^5 years of ^{26}Al decay. (**C**) Isopleths of ξ z^2 in effective temperature versus time space. These curves can be used to convert any combination of ξ and radius z into temperature and time. In the case of E44, ξ $z^2 = 5 \times 10^{-6}$ (heavy blue line).

or subsequent (possibly parent body) processing is indicated.

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19. For example, in a CAI the same size as E44 but with anorthite/(anorthite + melilite) = 0.05 rather than 0.25, the anorthite + melilite system would be reset when reaction progress variable = 0.4×10^{-3}, corresponding to 10 years at 1600 K or 10^7 years at 900 K.
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Supporting Online Material

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Structure of a γδ T Cell Receptor in Complex with the Nonclassical MHC T22

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γδ T cell receptors (TCRs), αβ TCRs, and antibodies are the three lineages of somatically recombined antigen receptors. The structural basis for ligand recognition is well defined for αβ TCR and antibodies but is lacking for γδ TCRs. We present the 3.4 Å structure of the murine γδ TCR G8 bound to its major histocompatibility complex (MHC) class Ib ligand, T22. G8 predominantly uses germline-encoded residues of its δ chain complementarity-determining region 3 (CDR3) loop to bind T22 in an orientation substantially different from that seen in αβ TCR/peptide-MHC. That junctionally encoded G8 residues play an ancillary role in binding suggests a fusion of innate and adaptive recognition strategies.

γδ T cells, like αβ T cells and B cells, generate a diverse repertoire of antigen-recognition receptors through somatic rearrangement of V, D, and J gene segments. This process generates a heterodimeric receptor composed of two chains, each encoding a variable (V) and constant (C) domain. It has been convincingly demonstrated that αβ and γδ T cells have different functional roles in the immune system (*1*), yet the identity of endogenous ligands for γδ T cells is unclear and little is known about the molecular basis of ligand recognition through their specific γδ TCRs. From the few defined γδ TCR ligands, it is apparent that γδ TCRs recognize a diverse array of antigens and that, like antibodies, they appear to recognize these antigens directly, distinguishing them from αβ TCRs that require antigen presentation by MHC [reviewed in (*2*)].

Early immunogenetic studies of αβ TCR and antibodies gave strong clues into the structural properties by which they would recognize ligand (*3*). In αβ TCR there is a concentration of diversity in the CDR3 (10^{15} unique junctions), relative to germline-encoded CDR1 and CDR2 derived from V-domain pairing (2500 pairs). In contrast, antibodies exhibit less CDR3 junctional diversity (10^{11} unique junctions) relative to the germline-encoded diversity of their CDR1 and CDR2 loops (90,000 pairs). Consistent with this, structural studies showed that the αβ TCR CDR3 primarily contact antigenic peptide, while CDR1 and CDR2 loops contact conserved helical portions of the MHC surface (*4*). Antibodies, although predominantly using CDR3, also make substantial use of CDR1 and CDR2 in recognizing a diverse antigenic repertoire.

A similar analysis of the γδ TCR repertoire indicates that they have the highest potential CDR3 diversity (10^{18}) but limited diversity conferred by pairing of germline-encoded V domains, with only ~7 Vγs and ~10 Vδs in the mouse (70 potential pairs) (*3*, *5*). CDR3 length distribution in γδ TCRs is more similar to antibodies than to αβ TCRs in that the CDR3δ loops are long and variable, and the CDR3γ loops are short and constrained (*5*). Given the long and potentially diverse CDR3δ of γδ TCR, it would seem likely that this loop is used directly for antigen recognition. However, many infection models involving γδ T cells show restricted V-gene usage (*6*), which suggests that γδ TCR specificity is determined by germline-derived V domains alone.

We have determined a 3.4 Å crystal structure of the γδ TCR G8 in complex with the nonclassical MHC Ib protein T22 (*7*, *8*). The G8 γδ heterodimer binds protein products of the T22 and T10 loci (95% homology), as do 0.09 to 0.6% of γδ T cells in the spleen and intraepithelial lymphocytes (IELs) of unstimulated mice (*9*, *10*). Both T22 and T10 have a canonical class I fold, except that the C terminus of the α2 helix is unraveled, disrupting the peptide-binding groove and exposing the underlying β-sheet platform (*11*, *12*). Binding measurements with refolded T22 (*9*) and the lack of a bound ligand in the structures of T10 (*12*) and T22 (*11*) confirm that G8 recognition of T10/T22 is direct and not dependent on antigen processing. The structure shows that the CDR loops, predominantly germline-encoded residues of the junctionally recombined CDR3δ, are directly used in γδ TCR recognition of an MHC ligand, resulting in a binding mode distinct from either antibody/antigen or αβ TCR/pMHC interactions.

Expression and structure determination of the γδ TCR and its ligand. The soluble G8 γδ TCR and its MHC ligand, T22, were expressed from baculovirus-infected insect cells, which produced a glycosylated G8 γδ TCR containing the canonical interchain di-

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sulfide (*13*). We confirmed binding of G8 to T22 by native gel shift and surface plasmon resonance (SPR). We crystallized the G8/T22 complex, collected a complete x-ray data set (Table 1), and determined a molecular replacement solution using the human γδ TCR G115 (*14*) and the unliganded structure of T22 (*11*) as search models. We subsequently refined the complex structure to 3.4 Å resolution (Table 1). There are two complexes in the asymmetric unit, forming a dimer along a Vδ interface (fig. S1). The Cγ and Cδ domains of one G8 γδ TCR (complex #2) exhibited partial disorder, and the electron-density map for these domains was poor, as reflected in elevated B values (Table 1) (*13*). All other parts of the structure are well resolved. The TCRs in the two respective complexes show a slight difference in orientation due to movement around a pivot point formed by the contact patch between CDR3δ and T22. This shift results in some minor variations in contact between the CDR1δ, CDR2δ, HV4δ, and CDR3γ loops and T22 between the two complexes. Because the individual components in the two complexes are identical to within the limits of our resolution [root mean square deviations (RMSD) of Cα individual G8 V domains and T22/β_2m are each 0.9 Å], we discuss complex #1 below and separately discuss the pivot and its implications in a later section.

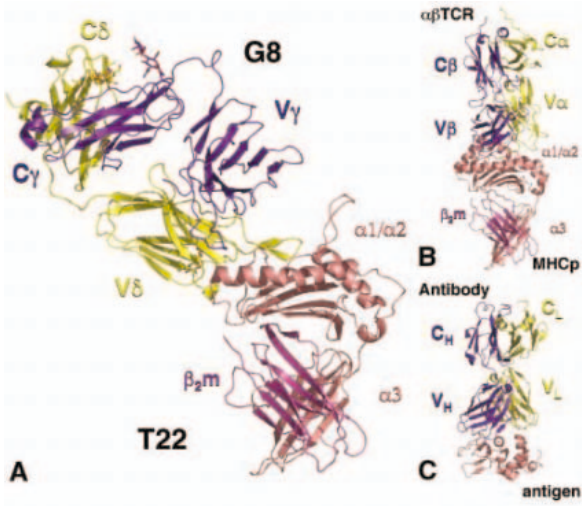

Fig. 1. Structure of the G8/T22 complex and comparison with ligand-recognition modes seen in αβTCR/pMHC and antibody/antigen complexes. (**A**) Ribbon diagram showing the overall backbone structure of the G8/T22 complex (only one of the two complexes in the asymmetric unit of the crystal is shown). The G8 γδTCR is shown in purple (γ chain) and yellow (δ chain), and T22 is shown in salmon (heavy chain) and magenta (β_2m). G8 binds T22 at a tilted angle, with an extended CDR3 loop from the δ chain forming extensive contact with T22's β-sheet platform and α1 helix. (**B**) The 2C αβTCR (α chain shown in yellow, β chain in purple) complexed with H2-K^b-dEV8 (heavy chain in salmon, β_2m in fuschia) (*25*). (**C**) Structure of the complex between the Fab of antibody HyHEL (heavy chain in purple, light chain in yellow) and hen egg-white lysozyme (shown in salmon) (*32*). All figures were produced with the Pymol software (*33*).

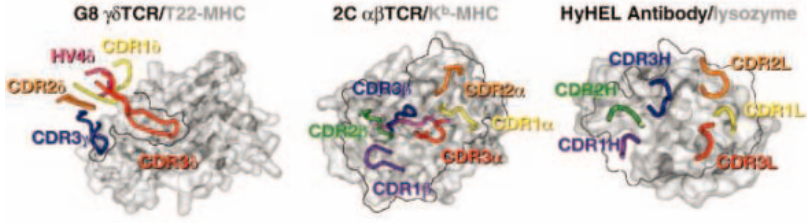

Fig. 2. Recognition footprints and location of CDR loops for γδTCR/MHC Ib, αβTCR/peptide-MHC, and an antibody/lysozyme complex. (**Left**) The CDR1δ (yellow), CDR2δ (orange), CDR3δ (red), HV4δ (hot pink), and CDR3γ (blue) loops of the G8 γδTCR are superimposed on a molecular surface of T22, with the ribbon backbone visible through the transparent surface. The remaining CDR loops of G8 do not participate in binding and therefore are not shown. The footprint of the G8 γδ TCR on T22 is shown as a black outline and occludes only a small portion of the T22 platform in comparison with that seen in αβTCR/pMHC (**middle**) (*25*) and antibody/antigen (**right**) (*32*). The black outlines of the three antigen receptors were traced around the perimeters of the respective molecular surfaces.

Table 1. Crystallographic statistics.

Data collection	
Space group	$P2_12_12_1$
Unit cell (Å) (a, b, c)	110.53, 113.05, 167.97
Source	ALS, 8.2.1
Resolution (Å) (last shell)	20–3.4 (3.52–3.40)
Unique reflections	28,542
Redundancy	5.9 (3.1)
Completeness (%)	96.9 (88.4)
I/σ (I)	23.9 (2.6)
R_{merge} (%)	5.2 (37.6)
Refinement statistics	
Resolution range	20–3.4 (3.52–3.40)
R_{free}	32.8 (43.2)
R_{cryst}	26.9 (34.1)
Average B factor (T22-1, T22-2, G8-1, G8-2)	91.4, 116.0, 87.7, 113.6
RMSD from ideality	
Bond lengths (Å)	0.0105
Bond angles (°)	1.80
Ramachandran plot (%) (favored, additional allowed, generously allowed, disallowed)	72.9, 24.3, 2.8, 0

Overall structure. The G8 γδ TCR is composed of four immunoglobulin (Ig) domains: two V domains, which encode the CDR loops, and two C domains, which are assembled into a quaternary structure similar to the previously published human γδ TCR heterodimer (*14*). T22 does not deviate appreciably from the previously described unliganded structure (*11*).

The structure shows that G8 uses its CDR loops to directly contact T22. Thus, the γδ TCR is not interacting with antigen through the germline-encoded framework regions of the V domains, as suggested by some functional studies (*6*, *15*), analogous to superantigen binding to αβ TCR (*16*). Rather, the γδ TCR engages ligand though its predicted antigen binding site. G8 binds T22 at a tilted angle that contrasts with the essentially parallel alignments of the long axes of the αβ TCR and pMHC when in complex (*17*, *18*). The length (12 residues) of the CDR3δ prevents a straight-on approach to T22 that would allow simultaneous close apposition of the CDR1 and CDR2 loops. This results in an almost side-on interaction mode primarily mediated by the δ chain (Figs. 1 and 2). Whereas αβ TCRs and antibodies often use all CDR loops in their recognition of peptide-MHC (pMHC) and protein antigen (Fig. 1, B and C, and Fig. 2) (*4*, *19*), the G8 γδ TCR uses the δ chain as the foundation of the interface with T22, with minor contact by CDR3γ (Figs. 1A and 2). The majority of G8 contact residues are contributed from a CDR3δ loop that binds in a cavity formed by T22's exposed β-sheet platform and α1 helix (Fig. 2).

The G8 γδ TCR interface with T22. The interface between G8 and T22 buries a total of 1936.1 Å^2 of surface area (Fig. 3A), comparable to the buried surface area (BSA) observed in most αβ TCR/pMHC and antibody/antigen interfaces (*19*). G8 contributes 996 Å^2 to the G8/T22 interface, with the overwhelming majority from the Vδ chain (886 Å^2 or

88.9%), and in particular, the CDR3δ (668 Å^2 or 67.1%) (Fig. 3B and table S1). CDR1δ, CDR2δ, HV4δ, and CDR3γ also make small contributions (Fig. 3B and table S1).

The region of buried surface in T22 (940.0 Å^2) is almost entirely within a cavity exposed by the absence of the H1 segment of the α2 helix and bordered by the intact α1 helix and the exposed α1/α2 β sheet (Fig. 3B). This cavity is in a similar location to where classical MHC molecules bind peptide. However, in T22, a 3–amino acid deletion, a disulphide bond between Cys^{110} and Cys^{133}, and the absence of peptide result in a partial unwinding of the α2 helix into a flexible loop (*11*), similar to that seen in the unliganded structure of the human MHC-like molecule, MIC-A (*20*). However, when MIC-A binds to its receptor NKG2D, the disordered segment refolds back into an α helix (*21*). In contrast, G8 binding does not engage the unwound portion of the α2 helix and instead targets the exposed groove (Fig. 3A).

The binding interface of G8's long CDR3δ loop spans from the edge of T22's α1/α2 β-sheet platform to the middle of the β sheet (Fig. 3A). The sideways binding orientation of G8 allows full extension of the CDR3δ loop into this cavity. The CDR3δ BSA is dominated by residues encoded by the two germline Dδ segments used in G8 receptor rearrangement (Fig. 3C), which suggests that the recognition of T22 by γδ TCRs (*10*) is mediated predominantly by restricted components of the CDR3δ loop. The amount of the buried surface area contributed by the CDR3δ loop alone (668 Å^2) is comparable to the entire BSA of some antibody/antigen and αβ TCR/pMHC complexes (table S5).

The bias of CDR3δ in the G8 binding mode contrasts with the majority of known αβ TCR/pMHC and antibody/antigen structure interfaces. There are examples of antibodies that have long CDR3 loops, such as those in the uncomplexed structure of the b12 neutralizing immunoglobulin G antibody against HIV-1 (*22*) and the recent structure of the shark single-domain antibody (IgNAR) in complex with its antigen, lysozyme (*23*). The long CDR3 of the shark IgNAR antibody, instead of extending away from the immunoglobulin domain, is pinned to the core by two disulfide bonds. This constraint reduces the effective length of the CDR loop, resulting in two smaller loops that, combined with the CDR1, compose the binding interface of the IgNAR receptor for lysozyme.

Interface residues. The G8/T22 interface was a well-resolved region of the complex structure, showing clear electron density for the majority of the contacting side chains (Fig. 4A). The CDR3δ loop interacts extensively with the β-sheet platform and truncated α1 helix of T22, undergoing an approximately 180° twist such that the C-terminal region of the loop contacts the α1 helix of T22 while the N-terminal side contacts primarily the β-sheet residues (Fig. 4B). The interface between the CDR loops of G8 and T22 is mediated mostly by hydrophobic interactions, with 84% of T22's contacts and 72% of G8's contacts being apolar residues (table S2 and Fig. 4B).

The primary contact residues in the CDR3δ loop are Thr^{92}, Trp^{93}, Ile^{95}, Gly^{98}, Tyr^{99}, Glu^{100}, and Leu^{101} (Fig. 4B and table S2). Thr^{92} is the result of N-nucleotide addition; Trp^{93} and Ile^{95} are encoded by the second reading frame of the D1 segment; Gly^{98}, Tyr^{99}, and Glu^{100} are encoded by the second reading frame of the D2 segment; and Leu^{101} is the result of P-nucleotide addition. Shin and co-workers have found that the majority of T22 tetramer-positive γδ TCRs characterized have both the Trp^{93} encoded by the germline Dδ1 segment and the repeating motif EGYEL encoded by the Dδ2 segment (*10*). However, they have also observed considerable flexibility in CDR3δ loop length, with G8 having an intermediate length compared with the CDR3δ loops of the T22 tetramer-positive γδ TCRs characterized. Our structure suggests that there are anchor residues in the CDR3δ loop contact interface, in particular Trp^{93} at the N-terminal end of the CDR3 loop, which is part of the extensive aromatic environment on the T22 exposed platform (Fig. 4B) and forms a hydrogen bond with Pro^{124} of T22 (table S3). Residues Gly^{98}, Tyr^{99}, Glu^{100}, Leu^{101}, and Thr^{103} appear to anchor the loop at its C-terminal end, reinforced by three G8/T22 hydrogen bonds (table S3 and Fig. 4B). How T22's cavity can accommodate longer CDR3δ loops in the context of these anchor positions (perhaps additional residues will loop out of the groove) awaits additional structure determinations.

Eight residues in T22 are within van der Waals contact distance to multiple (three or more) residues of G8, which suggests that they are potentially important interactions in the complex interface. Pro^{124} is the most prominent, with six interresidue contacts in the interface (Fig. 4C). Supporting the importance of Pro^{124} in this interface is the finding that an

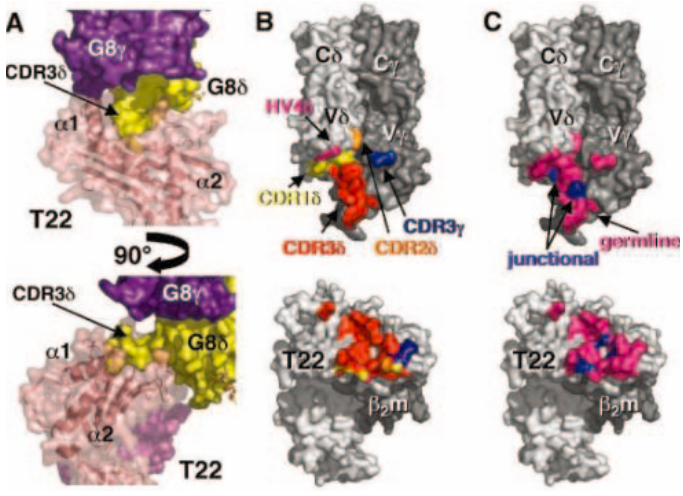

Fig. 3. Distribution of contact surfaces in the interface between the G8 γδ TCR and its MHC ligand T22. (**A**) Shape complementarity between the molecular surfaces of the G8 γδ TCR/T22 interface. The G8 γδ TCR is shown in purple (γ chain) and yellow (δ chain), and the T22 surface (shown in salmon) is transparent to show the ribbon diagram of the α helices and β sheet under it. Much of the interface involves the burying of G8's CDR3δ loop in a cavity over T22's β-sheet platform. (**B**) Breakdown of contacting surfaces between G8 and T22 colored by CDR loop. (Top) The CDR1δ (yellow), CDR2δ (orange), HV4δ (hot pink), and CDR3γ (blue) constitute a minority of the BSA of the G8 γδ TCR interface. The majority of the BSA of the binding interface is contributed by the CDR3δ loop (red). (Bottom) Molecular surface representation of the T22 molecule, with the respective contact sites of the CDR loops mapped onto the surface, color-coded by CDR contact as in the top panel. (**C**) Breakdown of the same contact surface between G8 and T22 colored by germline-encoded (shown in hot pink) versus junctionally generated amino acid residues (blue).

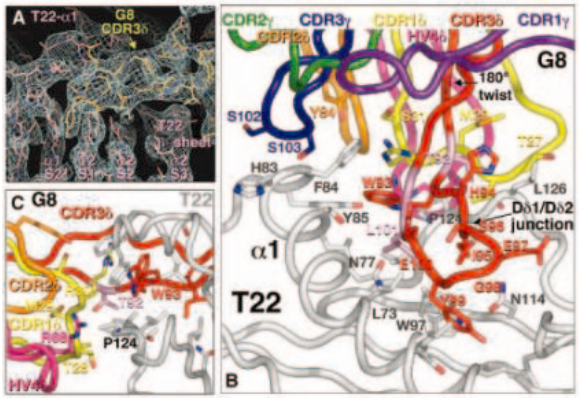

Fig. 4. G8 CDR loop interactions with T22. (**A**) Electron density of the G8/T22 interface showing the insertion of the CDR3δ loop in the T22 cavity (sigmaA-weighted $2F_o$-F_c). (**B**) Contacting amino acid residues within the G8 γδ TCR/T22 interface. The backbone of the CDR3δ loop (shown in red) undergoes a 180° twist. The contact residues of the CDR1δ (yellow), CDR2δ (orange), HV4δ (hot pink), and CDR3γ (blue) loops are also shown. (**C**) Side view showing the central location of the polymorphic T22 residue Pro124 in the G8 interface.

allelic form of the T10 molecule, $T10^d$, does not stimulate G8 (*24*). One of three allelic differences between $T10^d$ and T22 is a histidine instead of a proline at residue 124. This nonconservative substitution likely reduces binding stability and thus inhibits G8 stimulation. One of the Pro^{124} contact residues in the G8 CDR3δ loop, Thr^{92} (Fig. 4C), is encoded by a codon derived from junctional recombination. In other T22 reactive γδ TCRs, the amino acid(s) encoded by this junction vary (*10*), suggesting that the nature of the amino acid at the junction between the V and Dδ1 segment could modulate reactivity between T22 and T10 allelic forms.

Docking flexibility of G8 onto T22. The two G8/T22 complexes in the asymmetric unit have similar contact residues at the interface of the CDR3δ/T22 β sheet. However, extending outward from this interface, the two complexes differ by a relative rotation between the G8 TCRs (~5° rotation for Vδ, ~13° rotation for Vγ), resulting in a translation of approximately 2.4 Å for the δ chain and 6.4 Å for the γ chain when the distances between equivalent carbon-α atoms are measured (Fig. 5) (*13*). This shift alters the contacts formed between CDR1δ, CDR2δ, HV4δ, and CDR3γ loops and T22 in each TCR (table S2 and Fig. 5), suggesting that the CDR3δ loop acts as an anchored pivot point for G8 binding, with some flexibility in the interaction between the other CDR loops and T22.

This binding flexibility between G8 and T22, centered around anchored CDR3δ, is supported by the results of Shin and co-workers (*10*). These results demonstrate promiscuity of Vδ and Vγ chain usage in T22-specific γδ TCRs but conservation of the germline-encoded W…EGYEL motif in the CDR3δ chain. The commonality of this CDR3δ chain motif in T22+ tetramer-stained cells, despite usage of alternate Vδ and Vγ chains, supports the importance of the CDR3δ loop in ligand binding and further suggests that there is degeneracy in the perimeter contacts by the remaining CDR loops to T22. The hingelike flexibility around the pivot point stands in stark contrast to the fixed interactions seen in antibody/antigen and αβ TCR/pMHC complexes. In those cases, the relatively straight-on docking mode results in multipoint (i.e., multi-CDR) attachment of the receptor to the ligand, essentially rigidifying the intermolecular orientations between the two binding partners. In most cases, the αβ TCR CDR1 and CDR2 loops provide a perimeter of contacts with the MHC helices surrounding the CDR3 loops, and so far no variation has been seen in the docking angle of TCR to MHC in cases where multiple complexes exist in the asymmetric unit (*25*, *26*). Thus, the CDR3δ motif of G8 and other γδ TCRs (*10*) may be thought of as a somewhat autonomous binding entity that is presented by a variety of germline-encoded variable domain scaffolds without strong preference for particular CDR1 and 2 sequences.

Dimerization of G8. The two complexes in the asymmetric unit are dimerized through an extensive hydrogen-bonding network that involves the "a" strands of the Vδ domains (Fig. 6 and tables S4 and S5). The orientation of the two TCRs in the asymmetric unit is consistent with a biologically relevant dimer, where the membrane-bound complexes are on opposing cell surfaces. αβ T cells have been shown to require, minimally, oligomerization of their TCRs for activation (*27*); however, there is no crystallographic evidence of biologically relevant αβ TCR dimers. Activation of αβ T cells is also dependent on the expression of appropriate coreceptors such as CD4 or CD8. Some γδ T cells express the CD4 or CD8 coreceptors, yet it is unclear what role they play in γδ T cell activation (*28*). T10/T22–specific γδ T cells, including G8, are $CD4^-/CD8^-$, and therefore their activation appears to be coreceptor independent (*9*).

Dimerization of G8 on the cell surface during ligand binding could circumvent the need for coreceptor involvement and directly facilitate receptor oligomerization and subsequent T cell stimulation (*27*, *29*). However, we have not seen biochemical evidence of oligomerization of the soluble G8 TCR, although the dimerization affinity constant is likely very weak. The high concentration of G8 in the crystallization solution may exceed the dimerization affinity constant in a fashion similar to the elevation in effective concentration achieved by the two-dimensional restriction of the molecules on the cell surface.

Conclusion. The molecular basis of γδ TCR recognition has remained an enigma, as has the immunobiology of γδ T cells. Many characteristics of γδ T cells suggest that they participate early in the immune response, similar to other members of the innate immune system. These features include direct recognition of antigen and immediate effector outcomes such as cytokine release (*30*) and cytotoxicity (*31*). This contrasts with αβ T cells and B cells, both members of the adaptive arm of the immune system, which require a lengthier process of intracellular processing and presentation of antigen for initiation and maintenance of their effector functions. Yet, if γδ TCRs are part of the innate immune system, how do they use their combinatorial diversity in antigen recognition? The structure of G8 γδ TCR in complex with the MHC Ib

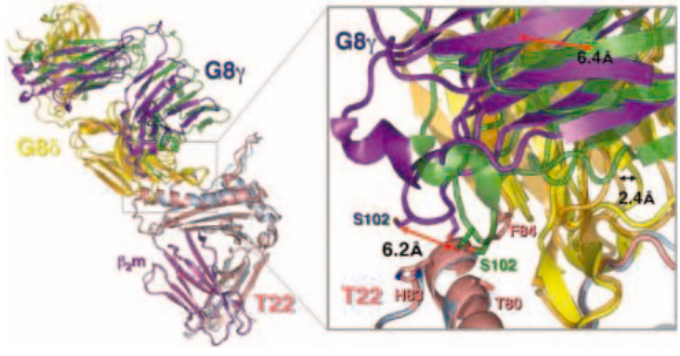

Fig. 5. Hingelike docking flexibility of the G8 TCR on T22. Superimposing the two G8/T22 complexes in the asymmetric unit on the T22 molecules reveals differences in G8 binding orientation centered around the CDR3δ pivot point. (**Left**) The two overall complexes superimposed. Complex 1 is shown as G8 in purple (γ chain) and yellow (δ chain), T22 in salmon (heavy chain) and fuschia (β_2m); complex 2 is shown as G8 in green (γ chain) and orange (δ chain), T22 in light blue (heavy chain) and steel blue (β_2m). (**Right**) Close-up of the Vγ and Vδ domain shifts of the G8 γδ TCR. The cores of the two Vδ domains shift by only 2.4 Å, whereas the two Vγ domains shift by 6.4 Å. For the displacement measurements, the distances between identical TCR residues in the superimposed complexes were measured (*13*).

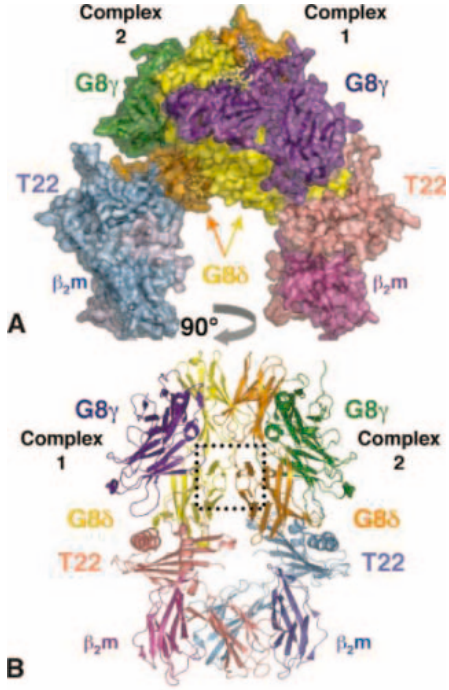

Fig. 6. A dimer of two G8 γδ TCR/T22 complexes. (**A**) Surface representation of the G8/T22 dimer present in the asymmetric unit. Complex 1 shows G8 in purple (γ chain) and yellow (δ chain) complexed to T22 in salmon (heavy chain) and fuschia (β_2m). Complex 2 shows G8 in green (γ chain) and orange (δ chain) complexed to T22 in light blue (heavy chain) and steel blue (β_2m). (**B**) The dimeric interface. The view in (A) is rotated 90° to show the dimer interface along the Vδ domains. The box highlights the location of the two "a" strands involved in the hydrogen-bonding network that constitutes the dimer; see fig. S1 for electron density of this interface.

molecule T22 suggests a convergence of innate and adaptive recognition strategies. G8, like αβ TCRs and antibodies, uses its CDR loops to bind its ligand, T22. However, G8 almost exclusively uses its genetically recombined CDR3δ loop to bind T22 from the side with degenerate contacts for the remaining CDRδ and CDR3γ loops, suggesting that the CDR3δ loop is the primary docking anchor in this recognition strategy. Moreover, the residues involved in the recognition interface are derived predominantly from germline-encoded Dδ segments, suggesting that there is, as previously hypothesized, a germline-encoded basis for T10/T22 recognition. The additional use of junctionally diverse residues in the interface allows for rapid coevolution of γδ TCRs with their ligands. This is an ideal strategy for an innate receptor, because there can be not only a long-term coevolution of the γδ TCR and its ligand over the lifetime of a species but also an immediate fine-tuning of the recognition of its particular ligand within an individual. In this way, the chemistry of the recognition interface between G8 and T22 can be thought of as a hybrid between innate and adaptive recognition solutions.

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Supporting Online Material

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Materials and Methods
Fig. S1
Tables S1 to S5

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REPORTS

The Real-Time Stellar Evolution of Sakurai's Object

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After a hot white dwarf ceases its nuclear burning, its helium may briefly and explosively reignite. This causes the star to evolve back into a cool giant, whereupon it experiences renewed mass ejection before reheating. A reignition event of this kind was observed in 1996 in V4334 Sgr (Sakurai's object). Its temperature decrease was 100 times the predicted rate. To understand its unexpectedly fast evolution, we have developed a model in which convective mixing is strongly suppressed under the influence of flash burning. The model predicts equally rapid reheating of the star. Radio emission from freshly ionized matter now shows that this reheating has begun. Such events may be an important source of carbon and carbonaceous dust in the Galaxy.

Helium-shell flashes play a vital role in the production of chemical elements in stars (*1*). They occur in all stars with initial masses between 1 and 8 times the mass of the Sun, during their asymptotic giant branch (AGB) phase. This phase ends with a drastic and rather sudden episode during which up to 90% of the star's mass is ejected into space. The remnant star evolves through a hot state toward the white dwarf cooling track, where nuclear burning ceases. Its radiation ionizes the ejected material and a planetary nebula forms. About one-quarter of these stars will undergo one final, very late He flash as a white dwarf, after nuclear hydrogen burning has ceased (*1*–*3*), in a unique event of nuclear flash–driven stellar evolution. The reincarnated star retraces its evolution and experiences renewed mass ejection.

The discovery of Sakurai's object (V4334 Sgr) in 1996 provided the first modern observations of this so-called very late thermal pulse (*4*). Its real-time evolution (possibly the fastest ever observed) provides a once-in-a-lifetime observational handle on the physics of convection and rapid nuclear burning. V4334 Sgr was the hot (10^5 K) central star of a hitherto undetected planetary nebula (*5*). Shortly before 1995, the helium layer reignited and V4334 Sgr was reincarnated as a "born-again" giant.

In our Galaxy, such an event is expected to occur once per decade (*6*). But V4334 Sgr was only the third observed case, after V605 Aql in 1918 (*7*) and possibly CK Vul in 1670 (*8*). Other events, such as the eruption of FG Sge (*9*), may have been missed in the crowded regions of the Milky Way. Hydrogen-poor [WC] central stars of planetary nebulae and the cooler hydrogen-deficient and carbon-rich R CrB stars (*10*) may be descendants of the born-again stars.

Computer simulations of the very late He-shell flash show that the small remaining H-rich envelope is convectively ingested into the He shell, resulting in an additional rapid H-driven nuclear flash burning. Initial models predicted that the stellar luminosity would increase, and temperature decrease, over a few hundred years (*1*, *2*). However, V4334 Sgr evolved at 100 times the predicted rate, suggesting neglected physics in the simulations (*11*, *12*). During 1998, increasing opacity by a dusty wind (*13*) rendered V4334 Sgr all but unobservable in the visual region. The mass

loss rate increased to 1.6×10^{-5} solar masses per year in 2001 (*14*). Indications for ionization were found in 2002 (*15*), suggesting that the star may be starting to reheat already.

To directly detect the renewed ionization, we observed Sakurai's object with the Very Large Array (VLA) on 5 February 2004. Optical images of O^{2+} emission were obtained using the focal reducer FORS1 on the ESO 8-m Very Large Telescope on 2 October 2002 (*16*). The image is shown in Fig. 1, with the VLA 8.6-GHz radio contours superposed. It shows the old planetary nebula (the extended shell) and a central radio source, marginally resolved, which has no O^{2+} counterpart. We identify this central source with the emerging ionized core around the reheating central star.

Radio observations carried out in November 1998 did not show emission near the central star (*17*). This suggests that a considerable increase in emission has recently occurred in this region and shows that the star has begun to reheat. Thus, we are witnessing the nascent stages of the formation of a new planetary nebula. The actual formation of such an H-poor planetary nebula has never before been observed. V605 Aql formed a compact H-poor emission nebula after the 1919–1924 outburst, but its formation was not observed; like V4334 Sgr, it became enshrouded in an envelope of dust that rendered it invisible to the astronomical tools of 1920 and was only recovered 60 years later (*18*).

The inset of Fig. 1 shows an image obtained in 2001 by the Hubble Space Telescope (HST) at a wavelength of 814 nm. The radio core of the newly formed ionized region is shown superposed as contours. The radio core has an integrated 8.6-GHz flux of 100 ± 30 μJy. The uncertainty is due to the variable nebular background. Gaussian fitting to the radio core gave a nominal deconvolved full width at half maximum (FWHM) size of 2.4×0.8 arc sec at a position angle of 170°. The radio core shows an indication of a double structure, but this has not been confirmed. Bipolarity is observed in the H-poor ejecta for the born-again objects A30 and A78 and in V605 Aql (*19*, *20*). Bipolarity could be caused in such very late He-flash objects by stellar rotation and/or a magnetic field.

We assume a distance to V4334 Sgr of 2 kpc (*21*, *22*). Early infrared spectra show a wind velocity of 670 km/s in the helium line at 1080 nm, relative to the central star (*23*). With a time line of 10 years, this velocity corresponds to a diameter of 1.4 arc sec, consistent with the observed radio feature.

The time scales for the stellar evolution are determined by the depth below the surface where the reignition takes place. To reproduce the large discrepancy in time scales, our revised stellar evolution models (*16*) parametrically include the buoyancy effect of rapid nuclear burning on convective turbulence in the He-shell flash zone. This reduces the convective mixing efficiency (*11*) and accelerates the evolution, because nuclear energy from fast proton capture is released closer to the stellar surface. The models reproduce the carbon isotope ratios and can explain the observed production of lithium (*16*, *24*). Models with suppressed mixing reproduced the fast cooling but predicted that V4334 Sgr would equally rapidly reheat (*12*, *25*).

Figure 2 shows our track of past and predicted future evolution. A good fit for the recent evolution is obtained if we assume that the reignition occurred in 1992 (the fast brightening in early 1995 can be explained by the rapid temperature evolution). The current detection of radio emission agrees with the predictions of rapid reheating. Continued monitoring over the next few years is essential to further test the evolutionary predictions. Confirmation of the effect of suppressed mixing has important implications for the physics of convective turbulence under the influence of rapid nuclear burning.

The newly ejected nebula consists almost exclusively of helium and carbon with very little hydrogen. We modeled these with the use of the photoionization code Cloudy (*16*). Abundance ratios were taken as those of the stellar atmosphere just before the obscuration (*24*), with C/He = 0.1 and H/He = 0.004. The radio flux, size, and line ratios were fitted with a hydrogen density $n(\mathrm{H}) = 36$ cm^{-3} and a dust-to-gas mass ratio of 3.9×10^{-2}. The electron temperature and density predicted by the model are shown in Fig. 3. The radial stratification of the most dominant ions causes a step-like decrease in the electron density. The N^+ diameter is smaller than the region of carbon ionization because of its higher ionization potential. The model predicts $N^+_{658.4\ \mathrm{nm}}/\mathrm{H}\alpha = 9.3$, in fair agreement with observations. The predicted ratio $O^+_{731.9+733.0}/N^+_{658.4\ \mathrm{nm}} = 8.0 \times 10^{-4}$ is in disagreement with the observed ratio of unity (*14*): The electron temperature is insufficient to excite the upper levels of the O^+ transitions. The detected O^+ lines may be shock excited, or the presence of large amounts of very small grains [as found in V605 Aql (*26*)] could lead to higher photoelectric heating than in our current model. Shock ionization is also not included in the model. Shocks at the observed wind velocity would lead to O^{2+} that is not observed, but a differential wind speed on the order of 100 km/s (as would

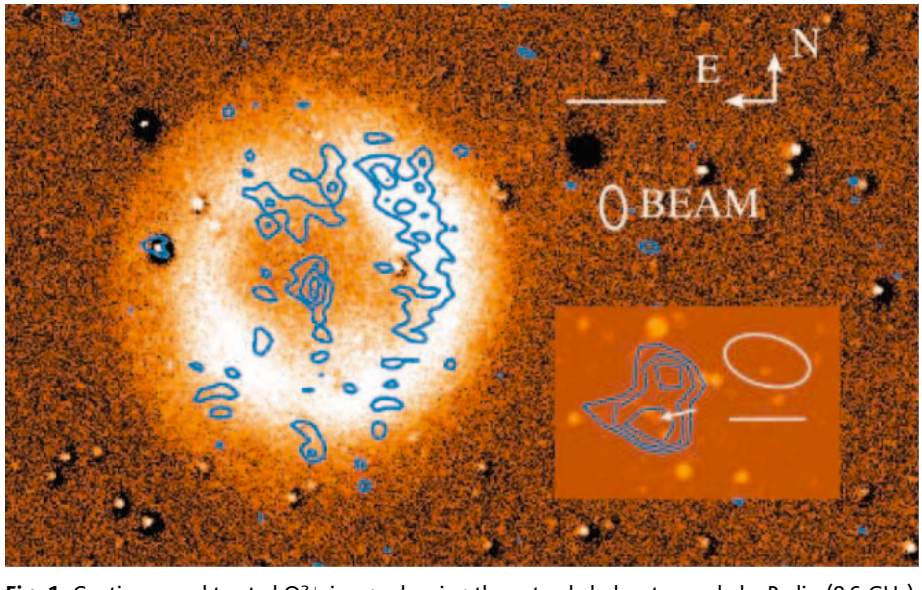

Fig. 1. Continuum-subtracted O^{2+} image showing the extended planetary nebula. Radio (8.6 GHz) contours are shown superposed at 30, 50, and 70 μJy per beam. A natural weighted map (beam of 4.2×2.4 arc sec indicated by the oval) is shown. Scale bar, 10 arc sec. (Inset) An HST I-band (F814W) image taken 29 August 2001. Sakurai's object (fainter of the two components, 0.2 arc sec apart) is indicated by an arrow. The superposed radio data show a uniform weighted map (beam of 2.2×1.3 arc sec, indicated by the oval) with contours at 25, 35, and 45 μJy per beam. The old planetary nebula is 41 arc sec in diameter; its brighter inner ring is 29 arc sec across. Scale bar, 2 arc sec.

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be expected if the star were reheating) could lead to O^+ formation.

The model predicts an ionized mass of 1.0×10^{-3} solar masses, mainly consisting of singly ionized carbon. Including the neutral mass within the ionized region yields 2×10^{-3} solar masses, where we use the mass ratio $X(C)/X(He) \sim 1$ corresponding to the model predictions (*11*). The dust mass is 1.85×10^{-4} solar masses. The total mass of the shell implies a mass loss rate of 2×10^{-4} solar masses per year. The observed mass loss rates have increased over time but reached only $\sim 10^{-5}$ in 2001 (*14*). This discrepancy may indicate that the shell is clumped, which would reduce our mass determination.

We find that Sakurai's object ejected about 5×10^{-4} solar masses of primary carbon, of which roughly 20% is located in the dust. Interstellar carbon dust comes mainly from AGB stars with C/O ratios above unity. In metal-rich populations, such as the inner Galaxy, this is reached in very few stars, and here Sakurai-type events may give an important contribution. V4334 Sgr shows a very high ratio of $^{13}C/^{12}C = 0.2$ (*27*) relative to the interstellar abundance ratio of 0.01. Together with novae (*28*), born-again giants may be the dominant ^{13}C source in the universe. Traces of this unique kind of mass ejecta may have been found in primitive meteorites. Isotopic analysis of presolar SiC A+B grains extracted from a sample of the Murchison carbonaceous meteorite are characterized by having $^{12}C/^{13}C < 10$. A subset of these grains also show enrichment of elements produced by slow neutron capture (s-process), and an origin in nuclear flash objects such as V4334 Sgr has been suggested (*29*). Our observations provide a quantitative estimate of the carbon mass lost in a born-again evolution and strengthen the possible link to presolar meteoritic grains.

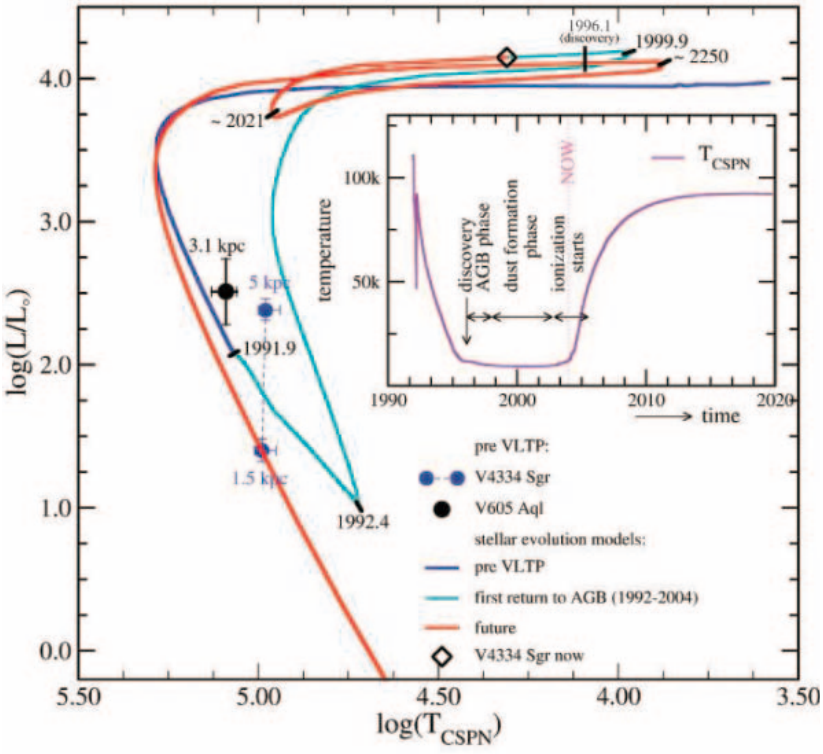
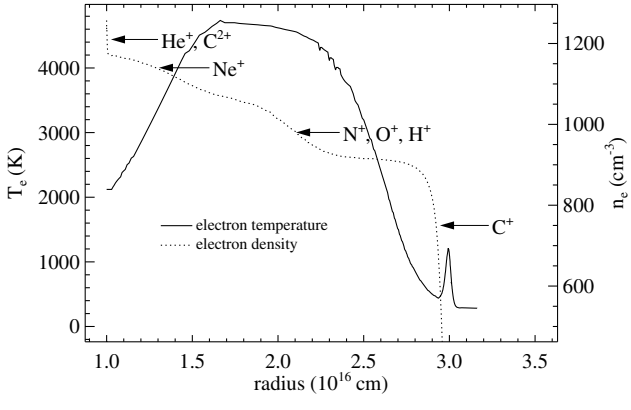


Fig. 2. Stellar model sequence of the past and future temperature and luminosity evolution of Sakurai's object. Two loops are predicted; the first loop corresponds to the hydrogen ingestion flash, and the second, much slower loop corresponds to the helium flash. Predicted times for past and future extrema are indicated. In this model, Sakurai's object is currently at the start of a fast temperature increase. The preflash positions of V4334 Sgr and V605 Aql are based on the ionization structure of their old nebulae (*5*, *7*).

Fig. 3. The physical conditions predicted by the Cloudy model. T_e, electron temperature; n_e, electron density.



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Formation of a Carbon-Carbon Triple Bond by Coupling Reactions In Aqueous Solution

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Formation of a carbon-carbon triple bond by coupling reactions usually takes place at high temperatures, in anhydrous media and anaerobic conditions. We describe the formation of a carbon-carbon triple bond at room temperature in an aqueous solution exposed to the atmosphere. Two ethylidyne ligands of a trimolybdenum cluster coupled spontaneously to form 2-butyne. This unexpected result demonstrates the plausibility of alkylidyne chain lengthening and metathesis processes under ambient, environmentally friendly conditions.

Numerous coupling reactions of transition-metal alkylidyne ligands (R–C) to form alkynes or alkyne ligands have been reported (*1*–*8*). However, in contrast to the analogous carbene (R_2C) transformations, this reaction class has yet to find widespread use in the selective preparation of polymers and fine chemicals (*9*). Alkylidyne coupling and alkyne metathesis have generally required inert atmospheres and anhydrous media, as well as elevated temperatures or photolytic activation (*10*). Trinuclear metal cluster compounds with ethylidyne, or other alkylidyne ligands R–C, are often used as models for the interaction between metal surfaces and organic moieties (*11*). Mechanistic investigations of such reactions have been hindered by their complex nature. The coupling product is often just one of several products formed simultaneously by parallel reaction paths.

Here we report two coupling reactions of a bis-ethylidyne cluster compound that proceed spontaneously at room temperature, in an aqueous solution exposed to the atmosphere. The two reactions investigated involve the complex ion $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3]^{2+}$ (**II**), where OAc is O_2CCH_3 (Fig. 1) (*12*). This complex ion is obtained by a one-electron oxidation of $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3]^{+}$ (**I**), which is the principal product of the reaction between $Mo(CO)_6$ and acetic acid (*12*). The two CCH_3 ligands in **II** cap a triangle of three molybdenum atoms, above and below the Mo_3 plane. The overall charge of the Mo_3 unit is 14+, corresponding to oxidation states IV, V, and V and four *d* electrons. The solid salts of **II** are stable for decades (*13*), but dissolving a salt of **II**, $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3](CF_3SO_3)_2$, in water at room temperature led to spontaneous decomposition within 3 to 4 hours. Product analysis revealed formation of free 2-butyne as well as two reduced trimolybdenum clusters: compound **I** and $[Mo^{IV}_3O_2(OAc)_6(H_2O)_3]^{2+}$ (**III**), in which the two capping CCH_3 ligands of **II** are replaced by two capping oxygen atoms (*14*).

We identified 2-butyne by gas chromatography–mass spectrometry (GC-MS) (*15*). Separation and titration of the metallic products revealed a quantitative reaction with the following stoichiometry: 5 $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3]^{2+}$ (**II**) + 2 $H_2O \rightarrow$ 4 $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3]^{+}$ (**I**) + $[Mo_3O_2(OAc)_6(H_2O)_3]^{2+}$ (**III**) + $CH_3C{\equiv}CCH_3$ + 4 H^+ (*16*). The oxidation states of the three Mo atoms in **I** are IV, IV, and V with five *d* electrons per cluster; in **III,** there are six *d* electrons and three Mo(IV) centers.

A simple electron count of the reaction shows that the products contain six more *d* electrons than the reactants. The source of these electrons are the two $[CH_3C]^{3-}$ groups of **II,** which are oxidized by the Mo_3 framework to $[CH_3C]^0$. After coupling of the two C_2 fragments, the volatile 2-butyne product leaves the system.

Four of the six electrons of one **II** ion were transferred stepwise to four other **II** ions, reducing them to four **I** ions. The remaining two electrons stayed in the framework of the intermediate Mo_3 cluster. Subsequently, two capping μ_3-oxo ligands, presumably from solution water molecules, were incorporated to form the bis-oxo capped complex **III**. The fact that this redox reaction is much slower in acidic solution (taking >24 hours in $[H^+]$ > 1 M) may indicate that it proceeds via μ-$H_3O_2^-$ bridges (*17*).

A second, related reaction occurs when **II** is introduced into aqueous 0.8 M HBr instead of pure water. In this case, a crystalline product precipitated from the solution after 10 to 12 days. The structure of this product, $[Mo_3Br_7(OAc)(H_2O)_2(CH_3C{\equiv}CCH_3)]$ (**IV**), was determined by a single crystal x-ray diffraction study (*15*, *18*) (Fig. 2). Compound **IV** forms with this stoichiometry: $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3]^{2+}$ (**II**) + 7 $Br^- \rightarrow$ $[Mo_3Br_7(OAc)(H_2O)_2(CH_3C{\equiv}CCH_3)]$ (**IV**) + H_2O + 5 OAc^-.

The coupling of the two ethylidyne groups is accompanied here by extensive changes in the coordination geometry and the chemical

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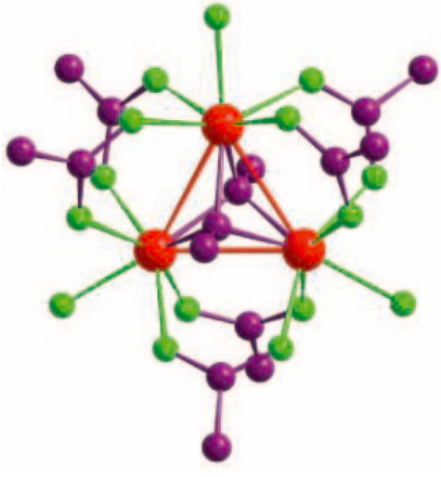

Fig. 1. Structure of **II**, $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3]^{2+}$. Red, molybdenum; green, oxygen; purple, carbon; hydrogen atoms are omitted for clarity. The two capping ethylidyne (CCH_3) groups are shown above and below the Mo_3 plane.

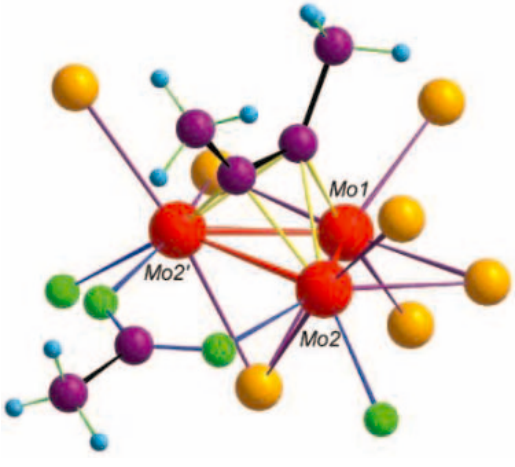

Fig. 2. Structure of **IV**, $[Mo_3Br_7(OAc)(H_2O)_2(H_3C{\equiv}CCH_3)]$. Red, molybdenum; green, oxygen; purple, carbon; blue, hydrogen; yellow, bromine. The 2-butyne ligand is shown above the Mo_3 plane. Mo1 and all carbon atoms reside on a crystallographic mirror plane that reflects Mo2 to Mo2′.

environments of the three metal atoms. Of the six bridging acetato ligands in the starting material **II**, only one is left in the product **IV**. Five acetate groups and one water ligand have been substituted by seven bromide ligands, of which four are in terminal positions, two in a doubly bridging position, and one in a capping position. The coupling product, the 2-butyne moiety, is not set free in this reaction, as it is in the first reaction, but remains coordinated to the Mo_3 framework in a μ_3-η^2 ($\perp$) fashion (*19*). The trinuclear complex **IV** resides on a crystallographic mirror plane that passes through Mo(1) and the two terminal bromine atoms attached to it. All carbon atoms of the 2-butyne and the acetate ligand are also bisected by this symmetry plane. The three molybdenum atoms form an isosceles triangle with a Mo(1)–Mo(2) distance of 2.5902(7) Å and a Mo(2)–Mo(2′) distance of 2.7412(7) Å. These Mo–Mo distances are much shorter than that found in **II**, 2.883(1) Å (*12*). The coordinated CH_3CCCH_3 ligand is not symmetric because of the unique μ_3-η^2 ($\perp$) binding mode. Thus, the two $(H_3)CCC$ angles are 119.2(5)° and 131.7(6)°, compared with 118(1)° and 131(1)° found in $[Fe_3(CO)_9(C_6H_5CCC_6H_5)]$ (*19*). The central C–C bond distance of 1.402(8) Å is also similar to the 1.41(2) Å distance found in the Fe_3 system.

The oxidation states of the molybdenum atoms in **IV** are II, III, and III with 10 *d* electrons in the system. The second reaction probably proceeds by an intramolecular mechanism, accompanied by a redistribution of the charge.

The common features of these two reactions offer some insight into the mechanisms of both. In order to enable coupling of the two remote ethylidynes of **II**, at least one of the CCH_3 ligands has to surmount the formidable barrier separating them. This barrier consists of a trimolybdenum plane with six carboxylates bridging the three edges of the Mo triangle and three water ligands at the axial positions. It is unlikely that acetate dissociation plays a role in the reaction, because complexes having the general Mo_3X_{17} structure have been shown to be kinetically inert to ligand exchange. The water ligands, however, are relatively labile (*20*). Dissociation of an axial water ligand may open a path for a flip-over of one CCH_3 group and its coupling with the second one to form a 2-butyne ligand, followed by its dissociation to form a free 2-butyne molecule in the first reaction. In the second reaction, such a dissociation of the 2-butyne ligand does not occur. It remains in its coordinated position in **IV** (Fig. 3).

These two reactions belong to the scarcely researched field of aqueous organometallic chemistry of high-valent metal complexes. The exploration of this and potentially other reactions that take place spontaneously in aqueous solution, at room temperature, may help to turn this somewhat esoteric field into a new frontier for organometallic chemistry. It may also open the door to cluster-based coupling and metathesis catalysts.

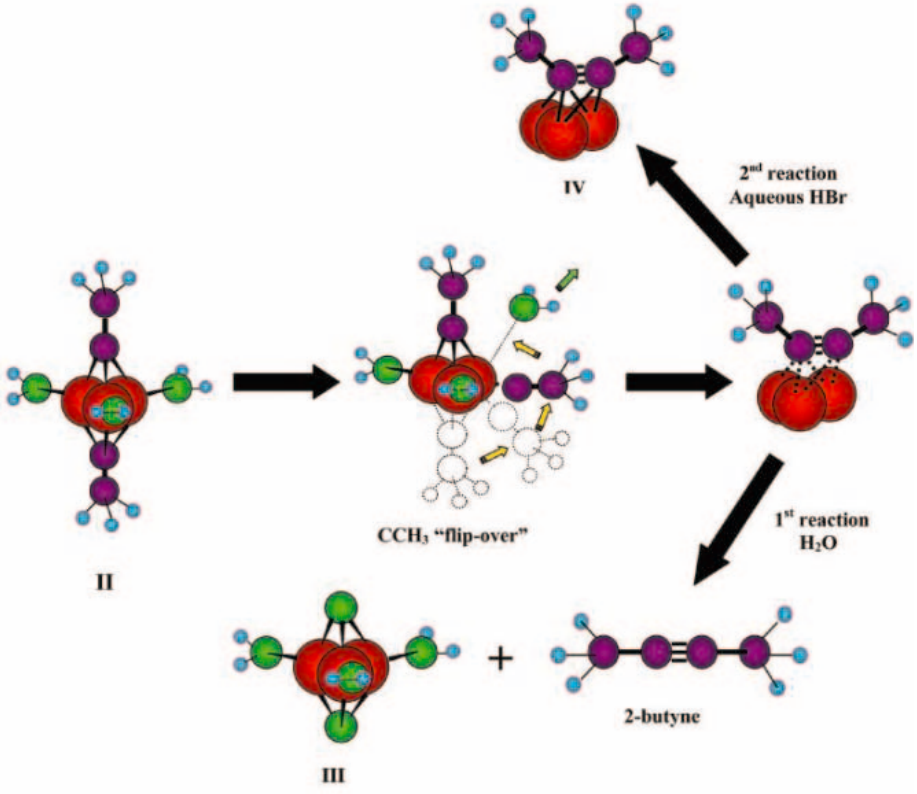

Fig. 3. Proposed common reaction path for the two coupling reactions. All bromide and acetate ligands are omitted for clarity.

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13. A crystalline sample of $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3](CF_3SO_3)_2$ that we prepared in 1976 remains unchanged to date (2005).
14. Compound **III** was previously prepared by a different method and fully characterized (*21*).
15. Materials and methods are available as supporting material on *Science* Online.
16. We dissolved 100 mg of $[Mo_3(CCH_3)_2(OAc)_6(H_2O)_3](CF_3SO_3)_2$ in 20 ml of H_2O in a 30-ml flask equipped with a rubber septum. The solution was kept at room temperature for 3 hours, during which the red color of **II** gradually changed to greenish brown. The gas above the solution was drawn by a syringe, in 1- to 2-ml aliquots, and injected into the gas chromatograph. The solution containing the products **I** and **III** was adsorbed on a Dowex 50W-X2 cation-exchange column. The 1+ ion **I** was eluted with H_2SO_4 (0.2 M) and the 2+ ion **III** by aqueous K_2SO_4 (0.5 M). Each fraction was oxidized by permanganate to Mo(VI), which was then passed through a Jones reductor into an acidified $Fe_2(SO_4)_3$ solution. The resulting Fe(II) was titrated by permanganate.
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18. Crystal data for $[Mo_3Br_7(OAc)(H_2O)_2(CH_3C{\equiv}CCH_3)]$ (**IV**): $C_6H_{13}Br_7Mo_3O_4$, M_r = 996.35, tetragonal, space-group $P4_2/nmc$, a = 17.6367(6) Å, c = 12.0818(8) Å, V = 3758.1(3) Å^3, Z = 8. For 1875 unique observed reflections collected at 123 K with $F^2 > 2\sigma(F^2)$, R = 0.0198.
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22. We thank A. Lifshitz for the GC-MS data and I. Felner for the magnetic measurements. Structural data for compound **IV** have been deposited in the Cambridge Crystallographic Data Centre under deposition no. 263471 and can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html.

Supporting Online Material
www.sciencemag.org/cgi/content/full/308/5719/234/DC1
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A Generalized Approach to the Modification of Solid Surfaces

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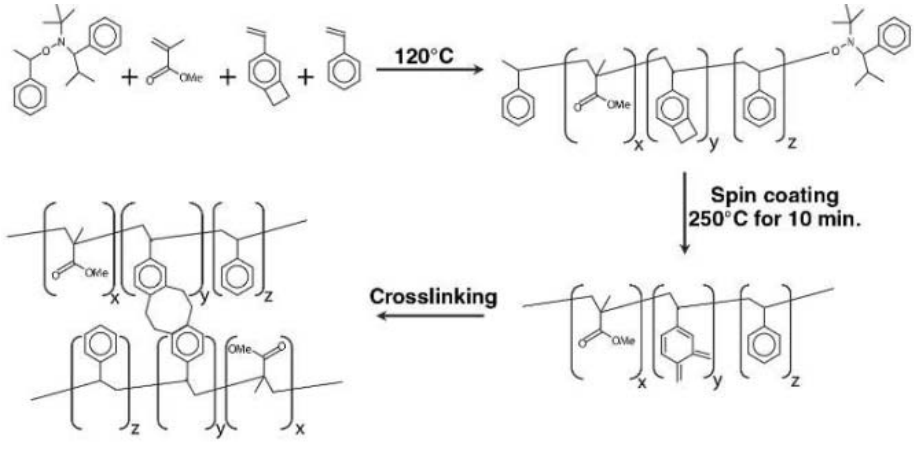
Interfacial interactions underpin phenomena ranging from adhesion to surface wetting. Here, we describe a simple, rapid, and robust approach to modifying solid surfaces, based on an ultrathin cross-linkable film of a random copolymer, which does not rely on specific surface chemistries. Specifically, thin films of benzocyclobutene-functionalized random copolymers of styrene and methyl methacrylate were spin coated or transferred, then thermally cross-linked on a wide variety of metal, metal oxide, semiconductor, and polymeric surfaces, producing a coating with a controlled thickness and well-defined surface energy. The process described can be easily implemented and adapted to other systems.

Controlling surface energies affords precise control over the surface and interfacial properties of a material, ranging from wetting to adhesion and, more recently, the orientation of nanoscopic structure in thin polymer films. Controlled interfacial interactions also find applications in synthetic (*1*–*6*) and biological systems (*7*) as surface-responsive materials (*8*). As a result of this rich array of applications, much attention has been placed on strategies to precisely tune interfacial and surface interactions of materials. Traditional approaches, however, rely on the use of surface-specific chemistries, such as aggressive ion beam techniques, self-assembly processes, or the chemical attachment of long-chain molecules to a surface. A notable drawback to these approaches is that they are not general and cannot be applied to a wide range of surfaces or substrates. For example, chlorosilane chemistries can easily be used on an oxide surface but cannot be used to modify the surface of gold or polymer films. Similarly, controlling the chemical composition with end-functionalized random copolymers allows exquisite control over surface energy, but the attachment of the chain requires time to have chain ends diffuse to the surface and very specific interactions and/or chemistries of the end group with the substrate (*1*). To date, there is no general approach to control interfacial and/or surface interactions. Here, we present a simple yet extremely versatile strategy, based on the cross-linking of ultrathin random copolymer films, for controlling and modifying interfacial and surface interactions.

This strategy takes advantage of the inherent versatility of random copolymers, which allows the surface energy or surface characteristics to be tuned by changing the chemical composition of the random copolymer. However, rather than relying on an inefficient and slow grafting procedure in which the chain end of the random copolymer diffuses to the surface and undergoes a very specific reaction with the surface, we used random copolymers containing a cross-linking group placed along the backbone. Using a simple, highly efficient cross-linking reaction, we obtained an insoluble, ultrathin film of the random copolymer that is more robust than an anchored random copolymer chain and, moreover, is independent of surface chemistry.

We studied random copolymers of styrene (S) and methyl methacrylate (MMA) with 2% reactive benzocyclobutene (BCB) functionality randomly incorporated along the backbone as an example. After spin coating, the BCB units in the BCB-functionalized polystyrene-*r*-poly(methyl methacrylate) copolymer [P(S-*r*-BCB-*r*-MMA)] can be thermally cross-linked to give a random copolymer network in which the thickness of the film is controlled by the concentration of the random copolymer solution spin coated onto the surface. The strength of the interfacial interactions can be changed by controlling the relative composition of S and MMA in the copolymer. In addition, the degree of cross-linking can be altered by changing the number of BCB units incorporated into the copolymer. The cross-linked film is insoluble and compatible with further processing. Removing the requirement of chemical attachment to the underlying substrate allows these cross-linked ultrathin films to be placed on most surfaces that can be coated. Even without chemical bonding to the surface, adhesive failure of the thin films was not observed (*9*) and the random copolymer architecture affords tremendous flexibility in circumventing this. In the case of PS and PMMA, previous studies have shown that a random copolymer containing 58% S that is end-anchored to the substrate produces a surface on which the interfacial interactions are balanced (*1*). Because BCB is chemically similar to S, the proportion of S/BCB/MMA in the copolymer used was 56/2/42. The random copolymer was prepared by nitroxide-mediated, living free radical polymerization (Scheme 1). To ensure sufficient cross-link density, molecular weights of between 20,000 and 100,000 were used with a typical sample having a number average molecular weight of 35,000 and a polydispersity of 1.18, which corresponds to an average of seven BCB units per chain. The polystyrene–poly(methyl methacrylate) block copolymer (PS-*b*-PMMA) used in these studies was prepared by anionic polymerization and had a weight average molecular weight of 88,000, a polydispersity of 1.03, and a 0.72 volume fraction of PS (*9*). In the bulk, the morphology consists of hexagonally packed cylindrical microdomains of PMMA in a PS matrix with a lattice spacing L_o = 34.1 nm. Thin films of PS-*b*-PMMA for which the micro-



Scheme 1. Synthesis and primary cross-linking reaction of the P(S-*r*-BCB-*r*-MMA), which has a composition of 56/2/42 for PS/BCB/PMMA.

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domain orientation can be controlled have tremendous potential as templates and scaffolds for the fabrication of nanoscopic materials (*10*–*16*).

A 0.3–weight percent (wt %) solution of P(S-*r*-BCB-*r*-MMA) in toluene was spin coated onto a silicon wafer to yield films ~11.1 nm thick. The coated substrates were heated under a nitrogen atmosphere to either 200° or 250°C [well above the glass transition temperatures of PS (100°C) and PMMA (115°C)] for different periods of time to investigate the cross-linking of the random copolymer. Subsequently, the films were thoroughly rinsed with toluene to remove material that was not cross-linked and the thickness of the insoluble cross-linked random copolymer remaining on the substrate surface was measured by ellipsometry (Fig. 1). The thickness of the film increased as time increased, and after 4 hours at 200°C or 10 min at 250°C, the film thickness reached a constant value of 10.4 nm. The final value of 10.4 nm is ~6% thinner than the initial film thickness of 11.1 nm, reflecting the volume shrinkage associated with cross-linking. As the data in Fig. 1 show, the rate of cross-linking decreases as temperature decreases, and, from independent measurements, ~150°C sets an effective lower limit on the efficiency of the BCB cross-linking. This lower temperature limit does prevent us from coating crystallizable polymers with low melting temperatures. Although it was possible to coat these materials, volume contractions and marked changes to the surface topography that occur during crystallization damaged the coating.

To demonstrate the effectiveness of these cross-linked random copolymer mats in controlling surface energy, thin films (~10 nm) of PS and PMMA homopolymers were spin coated onto Si substrates coated with cross-linked P(S-*r*-BCB-*r*-MMA) films (10 min at 250°C) (Fig. 1). The homopolymer films were then heated to 170°C for 3 days, allowing the homopolymer films to dewet. The shapes of the edges of the dewetted PS and PMMA films were determined from the height profiles with scanning force microscopy. From the shape, the contact angles of the PS and PMMA on the P(S-*r*-BCB-*r*-MMA) were determined and are plotted in Fig. 2 as a function of the thickness of the P(S-*r*-BCB-*r*-MMA) on the surface. In the case of PS, the contact angle is initially ~13°, decreases to 9.0° as the thickness of the P(S-*r*-BCB-*r*-MMA) film increases to ~5 nm, and then remains constant for thicker P(S-*r*-BCB-*r*-MMA) coatings. PMMA, on the other hand, has a contact angle of ~5° that increases to 10.1° when the P(S-*r*-BCB-*r*-MMA) coating is ~5 nm, and remains constant for thicker coatings of the random copolymer. Thus, for P(S-*r*-BCB-*r*-MMA) coatings, 5 nm or thicker, the contact angles for PS and PMMA are essentially the same. The changes in the contact angles for the PS and PMMA can be understood when one considers the increasing number of P(S-*r*-BCB-*r*-MMA) cross-links, the increasing thickness of the random copolymer film, and the decreasing penetration of the homopolymer to the underlying oxide layer of the substrate. The constant contact angles obtained for PS and PMMA on random copolymer films thicker than 5 nm (cross-linked for 10 min at 250°C) can be used to evaluate the interfacial energies from Young's equation:

$$\gamma_{if} = \gamma_f - \gamma_i \cos\theta_{if} \quad (1)$$

where γ_i and γ_f are the surface tensions of the homopolymer and the random copolymer, respectively, and θ_{if} is the contact angle of the homopolymer on the cross-linked random copolymer mat. From previous studies (*1*), $\gamma_S = 29.9$ erg/cm^2 for PS, $\gamma_M = 30.02$ erg/cm^2 for PMMA, and $\gamma_f = 29.95$ erg/cm^2 for a neutral random copolymer brush that has 58% of S. From the contact angles of 9.0° and 10.1° for PS and PMMA, respectively, $\gamma_{Sf} = 0.415$ erg/cm^2 and $\gamma_{Mf} = 0.397$ erg/cm^2. These are the same to within experimental error and, consequently, the interfacial interactions are balanced.

Films of cross-linked P(S-*r*-BCB-*r*-MMA), 7 nm in thickness after heating to 250°C for 10 min under nitrogen, were prepared on a variety of substrates, including metals (Au and Al), semiconductors (Si, SiOx, and Si_3N_4), and polymers [Kapton (aromatic polyimide) and polyethylene terephthalate (PET)]. These substrates present a wide range of interfacial interactions and chemistries that are not compatible with any one functionality. Notably, Au surfaces are difficult to modify in a robust manner with thiol monolayers, because typical annealing temperatures of the block copolymers are ~170°C, which is much greater than the dissociation temperature of the Au-thiol bond. Shown in Fig. 3, A and B, are water droplets on a silicon wafer and Au substrate, respectively, coated with 7-nm layer of cross-linked P(S-*r*-BCB-*r*-MMA). The contact angles are 76.2° and 76.1°, respectively. Without the random copolymer layer, the contact angles are 17.4° for SiO_2 (Fig. 3C) and 63.4° for Au (Fig. 3D). As shown, the surface energies of the hydrophilic (silicon oxide) and hydrophobic (Au) surfaces have been changed and are identical.

A P(S-*b*-MMA) diblock copolymer film, ~33 nm or L_o in thickness, was spin coated onto Au-patterned Si substrates, prepared by thermal evaporation with a mask (a Cr adhesion layer was needed to adhere the Au to the substrate), without (Fig. 3E) or with (Fig. 3F) a 7-nm layer of cross-linked P(S-*r*-BCB-*r*-MMA), and annealed for 24 hours under vacuum at 170°C. Shown in Fig. 3, G to J, are scanning force microscopy phase images of the block copolymer film on the bare Au (Fig. 3G) and silicon oxide–coated (Fig. 3I) portions of the substrates and the corresponding sections (Fig. 3, H and J) coated with a 7-nm-thick layer of cross-linked P(S-*r*-BCB-*r*-MMA). The surfaces coated with P(S-*r*-BCB-*r*-MMA), on which the interfacial interactions are balanced (*1*, *17*), produce films in which the cylindrical microdomains are oriented normal to the surface, regardless of the underlying substrate. However, on the bare Au surface poor control over the microdomain orientation was achieved (Fig. 3G), and on the silicon oxide surface the microdomains orient parallel to the surface (Fig. 3I) and show the classic island and hole topography. In agreement with the PS and

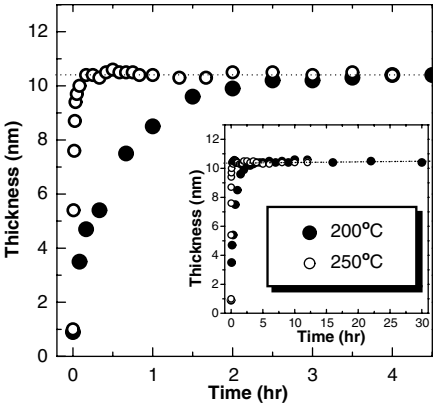

Fig. 1. The thickness of the P(S-*r*-BCB-*r*-MMA) film as a function of the reaction time at 200° and 250°C after rinsing with a good solvent.

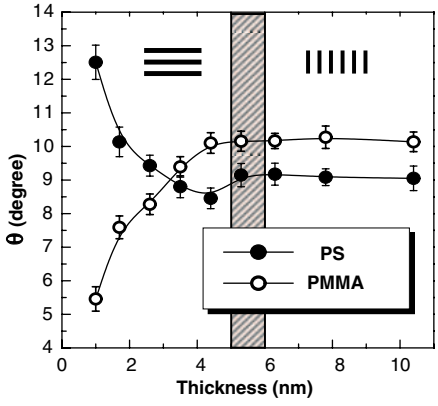

Fig. 2. Contact angles for PS and PMMA as a function of P(S-*r*-BCB-*r*-MMA) film thickness after annealing at 250°C for 10 min, when the copolymer is fully cross-linked and there is no change in film thickness upon rinsing with a good solvent. Homopolymer films (~10 nm thick) were spin coated onto the P(S-*r*-BCB-*r*-MMA) modified substrates and annealed at 170°C for 3 days. For clarity, a shaded region at 5.5 ± 0.5 nm marks the P(S-*r*-BCB-*r*-MMA) thickness where a change in the microdomain orientation (from parallel to perpendicular) was observed for P(S-*b*-MMA) copolymer. Error bars show mean ± SD.

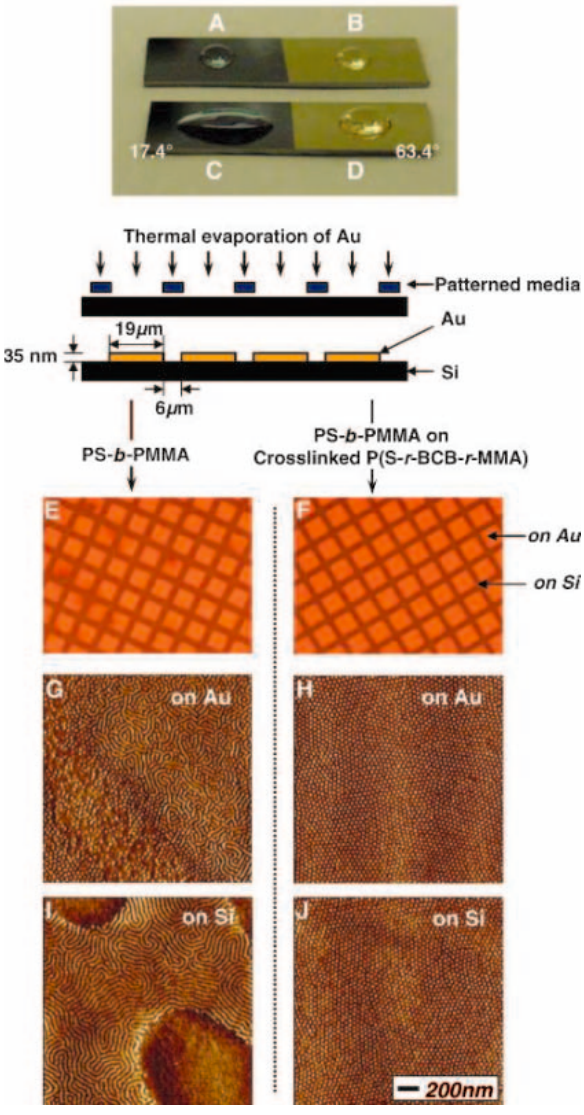

Fig. 3. Water droplets on the Si (**A**) and Au (**B**) substrates coated with a 7-nm-thick cross-linked film of P(S-*r*-BCB-*r*-MMA) and on bare Si (**C**) and Au (**D**) substrates without the copolymer. A P(S-*b*-MMA) copolymer spin coated and annealed for 1 day at 170°C onto the Au-patterned Si substrate prepared by thermal evaporation with a patterned mask without (**E**) or with (**F**) cross-linked random copolymer mat. Scanning force microscopy phase images of a P(S-*b*-MMA) copolymer on the bare Au (**G**) and Si (**I**) substrates and on the Au (**H**) and Si (**J**) substrates with cross-linked random copolymer mat. Block copolymer films are ~33 nm (or ~1 L_o) thick and the *z* range of phase images is between ~6° and ~10°.

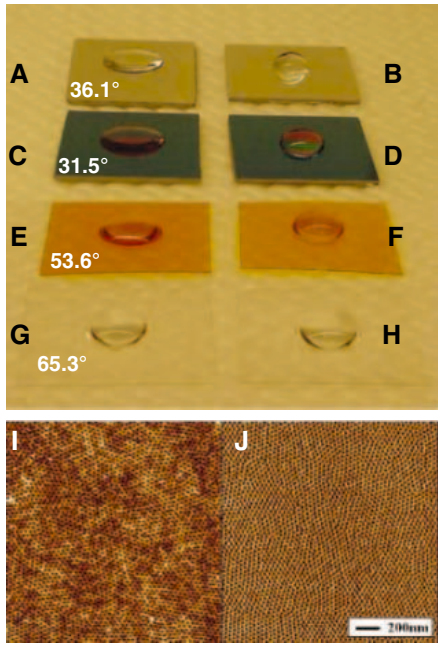

Fig. 4. Water droplets on bare Al (**A**), Si_3N_4 (**C**), Kapton (**E**), and PET (**G**) substrates. The contact angles range from 31.5° to 65.3° depending on the substrate. Water droplets on each substrate coated with 7-nm layer of cross-linked P(S-*r*-BCB-*r*-MMA), however, are the same 76° ± 0.3° (**B**, **D**, **F**, and **H**). Scanning force microscopy height (**I**) and phase (**J**) images of a ~33-nm-thick film of P(S-*b*-MMA) copolymer prepared on substrates coated with a film of cross-linked random copolymer mat regardless of the underlying substrate. PMMA was selectively removed by exposing the film to ultraviolet radiation and rinsing with acetic acid as a good solvent for PMMA. This nanoporous PS film constitutes a template or scaffold for the fabrication of nanostructured materials.

PMMA dewetting studies, a layer of P(S-*r*-BCB-*r*-MMA) at least 5 nm in thickness was required to control the microdomain orientation (indicated by the vertical shaded region in Fig. 2). In addition, the thin film of cross-linked P(S-*r*-BCB-*r*-MMA) protects the Cr adhesion layer from oxidation. Many defects are seen in the untreated patterned surface (compare Fig. 3, E and F).

The versatility of the cross-linked random copolymer mat approach is demonstrated in Fig. 4, which shows water droplets on bare Al (Fig. 4A), Si_3N_4 (Fig. 4C), Kapton (Fig. 4E), and PET (Fig. 4G) substrates. The contact angles range from 31.5° to 65.3° depending on the substrate. When these substrates are coated with a 7-nm layer of cross-linked P(S-*r*-BCB-*r*-MMA), however, all of the contact angles to within one standard deviation (±SD) are the same at 76° ± 0.3° (Fig. 4, B, D, F, and H). L_o thick films of P(S-*b*-MMA) spin coated onto each of the substrates coated with the 7-nm-thick cross-linked random copolymer mat showed an orientation of the cylindrical microdomains normal to the surface regardless of the underlying substrate. When we exposed these films to ultraviolet radiation, the PS was cross-linked and the PMMA was degraded; after rinsing with acetic acid, pores were produced (as evidenced by scanning electron microscopy) that extend from the film surface to the underlying substrate. In both the height (Fig. 4I) and phase images (Fig. 4J), cylindrical pores with 20-nm diameters are seen in the PS matrix (with a 34.1-nm spacing). Even though the random copolymer mat contains MMA, the integrity of the underlying random copolymer film is maintained by virtue of the cross-linking and the block copolymer template remains intact.

We have developed a method in which the interfacial interactions of a surface can be easily manipulated in a rapid, robust manner through the use of a thin, cross-linked random copolymer film. This technique does not require specific chemical reactions or interactions with the surface. Because the film is cross-linked, it is resistant to solvents and forms a robust coating on the surface. The effectiveness in controlling the interfacial interactions was demonstrated with thin diblock copolymer films on a wide range of substrates on which the orientation of the microdomains was demonstrated.

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Estimating Duration and Intensity of Neoproterozoic Snowball Glaciations from Ir Anomalies

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The Neoproterozoic glaciations supposedly ended in a supergreenhouse environment, which led to rapid melting of the ice cover and precipitation of the so-called cap carbonates. If Earth was covered with ice, then extraterrestrial material would have accumulated on and within the ice and precipitated during rapid melting at the end of the glaciation. We found iridium (Ir) anomalies at the base of cap carbonates in three drill cores from the Eastern Congo craton. Our data confirm the presence of extended global Neoproterozoic glaciations and indicate that the duration of the Marinoan glacial episode was at least 3 million, and most likely 12 million, years.

The Snowball Earth hypothesis (*1*–*3*) states that the Sturtian [about 710 million years ago (Ma)] and Marinoan glaciations (about 635 Ma) were of global extent and lasted for several million years each. A variation of this hypothesis, called the Slushball Earth, requires milder conditions without substantial equatorial sea ice (*4*, *5*). The Snowball Earth glaciations would have ended abruptly in a greenhouse environment, whereas the Slushball would have experienced a slower deglaciation. Silicate weathering and photosynthesis, which are the major sinks for CO_2 at present, would have been inhibited by the ice that covered both continents and oceans, and huge amounts of CO_2 could have accumulated in the atmosphere. Greenhouse gases, particularly large amounts of CO_2 (up to ~0.12 bar), would have been needed to overcome the high albedo caused by the glaciation and would have caused rapid melting (*6*). This scenario has led to an estimate for the duration of a Snowball glaciation of ~4 million to 30 million years (My), under the assumption of a modern rate for CO_2 outgassing from subaerial volcanism, with no air-sea gas exchange, lower solar luminosity in the Neoproterozoic, and reduced pelagic deposition of carbonate to reduce the release of volcanic CO_2 at convergent margins (*7*). This estimate is in broad agreement with a >6- to >10-My duration derived from the $\delta^{13}C$ isotopic excursion in the Otavi (North Namibia) cap carbonates (*2*). Multiple magnetic polarity reversals within the Elatina Formation (South Australia) suggest a minimum time duration of several hundred thousand to 1 million years for the Marinoan glacial epoch (*8*).

If Earth was indeed covered by ice for long periods, extraterrestrial material would have accumulated on and within the ice and would have been precipitated at the base of the cap carbonates when the ice melted. To determine whether such an extraterrestrial signal is present at the base of the cap carbonates, we studied three drill cores that intersected diamictite/cap carbonate boundaries from the Lufilian tectonic arc in the Democratic Republic of the Congo and Zambia of the Eastern Congo craton (Fig. 1) (*9*).

We measured the concentrations of 43 elements by neutron activation analysis and x-ray fluorescence spectrometry, and we measured the Ir content by multiparameter coincidence spectrometry after neutron activation (*10*). Ir anomalies were found at the base of all cap carbonates after the Marinoan and Sturtian glaciations in the Nguba and Kundelungu Groups at Kipushi, as well as after the Sturtian glacial deposits in the Nguba Group at Chambishi. Ir and other platinum-group elements are typical proxies for extraterrestrial material, in which they are much more abundant than in Earth's upper mantle and crust. The average Ir concentration in cosmic matter is about 4.6×10^5 parts per thousand (ppt) (*11*), which is greater by a factor of about 10^4 than in crustal terrestrial rocks.

The accretion of extraterrestrial material during the Neoproterozoic is thought to have been dominated by the delivery of interplanetary dust particles (IDPs) over a time scale of millions of years (*12*). IDP accretion rates vary over periods ranging from a few thousand years (*13*) to millions of years (*14*). During glaciations, IDPs, as well as extraterrestrial matter from asteroids and comets that struck the Earth during that time (*9*), would have accumulated on the ice sheet.

Substantial Ir anomalies up to almost 2 parts per billion (ppb) mark the base of the cap carbonate deposits (Fig. 2) (*9*). Before ascribing an extraterrestrial origin to the Ir, possible terrestrial sources, including reduced sedimentation rates, increased meteor ablation rates, accretion of extraterrestrial material or of terrestrial dust (such as volcanic airborne particles), and anoxic conditions connected with sulfide precipitation in seawater need to be considered. Our geochemical data clearly indicate that the (substantial) Ir anomalies at the base of the cap carbonates are derived from extraterrestrial sources, whereas the other (smaller) Ir anomalies disappear or are greatly diminished when ratios with other elements are used [see (*9*) for detailed considerations]. Thus, these variations can be attributed to changing deposition rates or to the dissolution of an extraterrestrial signal caused by the addition of sediment.

Further confirmation that the Ir anomaly at the Kipushi Petit Conglomerat/cap carbonate transition (KPCCT) is of extraterrestrial origin comes from Cr/Ir and Au/Ir ratios of 2.9×10^3 and 0.11, respectively. These values are similar to those of C1 chondrites (carbonaceous chondrites type 1) (5.5×10^3 and 0.29, respectively) (*15*). Cr/Ir and Au/Ir ratios from other samples show higher values ranging from 8.4×10^4 to 1.8×10^6 and 1.1 to 80, respectively (Fig. 2).

In contrast to the sharp Ir anomaly at the KPCCT, the Nguba Group Ir anomaly extends over a broader interval, between 236.91 and 237.93 m, with a maximum of 648 ppt of Ir at 237.93 m. The integrated Ir concentrations are, however, as high as those at the other location. This Ir anomaly does not occur exactly at the Kipushi Grand Conglomerat/cap carbonate transition (KGCCT), but 0.63 m above, at 238.56 m in the cap carbonate succession (Fig. 2). Ir/element ratios with Fe, Cs, and Al, compared with the Grand Conglomerat and cap carbonate succession, are much higher in

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the Ir-enriched interval. Ni/Ir ratios of 1.1×10^4 at 236.91 m and 2.6×10^4 at 237.93 m, respectively, are similar to the C1 chondritic value of 2.3×10^4 and are distinctly lower than the values throughout the cap carbonate succession. Also, Au/Ir and Cr/Ir ratios of 0.46 and 1.05×10^4, respectively, at 237.93 m are also similar to the corresponding chondritic values of 0.29 and 0.55×10^4 (*15*), whereas ratios below and above the Ir anomaly are comparatively much higher. No indicators exist for hydrothermal, hydrogenous, or volcanic influences or for strong anoxic or reducing conditions that could produce an Ir anomaly. Thus, this enrichment was also likely caused by admixture of extraterrestrial material. The fact that the Ir anomaly after the Sturtian glaciation is characterized by a broader peak of high Ir, Co, Cr, and Ni values near the base of the cap carbonates could be the result of a transgression and slightly delayed deposition of the cap carbonates (*3*).

In the Chambishi core, the Ir anomaly is located closer to the top of the Sturtian glacial deposits (only 9 cm above the top of the Grand Conglomerat) at 147.72 m than in the Kundelungu succession. The Ir anomaly at the Chambishi Grand Conglomerat/cap carbonate transition (CGCCT) is distinguished from the other Ir anomalies by an Ir/Fe ratio that is twice as high (Fig. 2). Changes in debris rainout during the glaciation caused by basal melting of ice sheets, floating glacier tongues, or episodic migration of calved icebergs into regions of warmer water (*16*) would affect the relative abundances of Fe and Ir but not their ratio. Thus, the occurrence of new source material in the glacial deposits may indicate warming periods during the glacial episode. The Ir/Al ratio and element/Cs ratios for Ce, Co, Cr, Eu, Fe, Hf, Sc, Ta, and Th at all Ir anomalies in this succession show values that are similar to the background values, except the Ir/Cs ratio at the Ir anomaly at 170.70 m, which is twice as high as that at the other Ir anomalies. However, the carbonate succession over the CGCCT Ir anomaly shows very high Ir/Fe, Ir/Al, and Ir/Cs ratios and low Co/Ir and Cr/Ir ratios, as well as Ir abundances of 45 to 60 ppt. This is very high, if we assume that cap carbonates precipitated very rapidly (*17*–*19*). Furthermore, at 144.96 m, strong evidence for the input of volcanogenic material is signaled by the very high element/Al ratios of Th, Ta, Hf, K, and Ti, which indicate the inclusion of a silicic volcanic component and/or a Hawaiian-like volcanic component (*20*, *21*) (Fig. 2). However, it does seem that the deposited material was mostly extraterrestrial. It was probably not deposited at once but rather swept into the sea from uplands after a long glaciation, which was recorded for the Sturtian period in the Nguba Group succession from Kipushi.

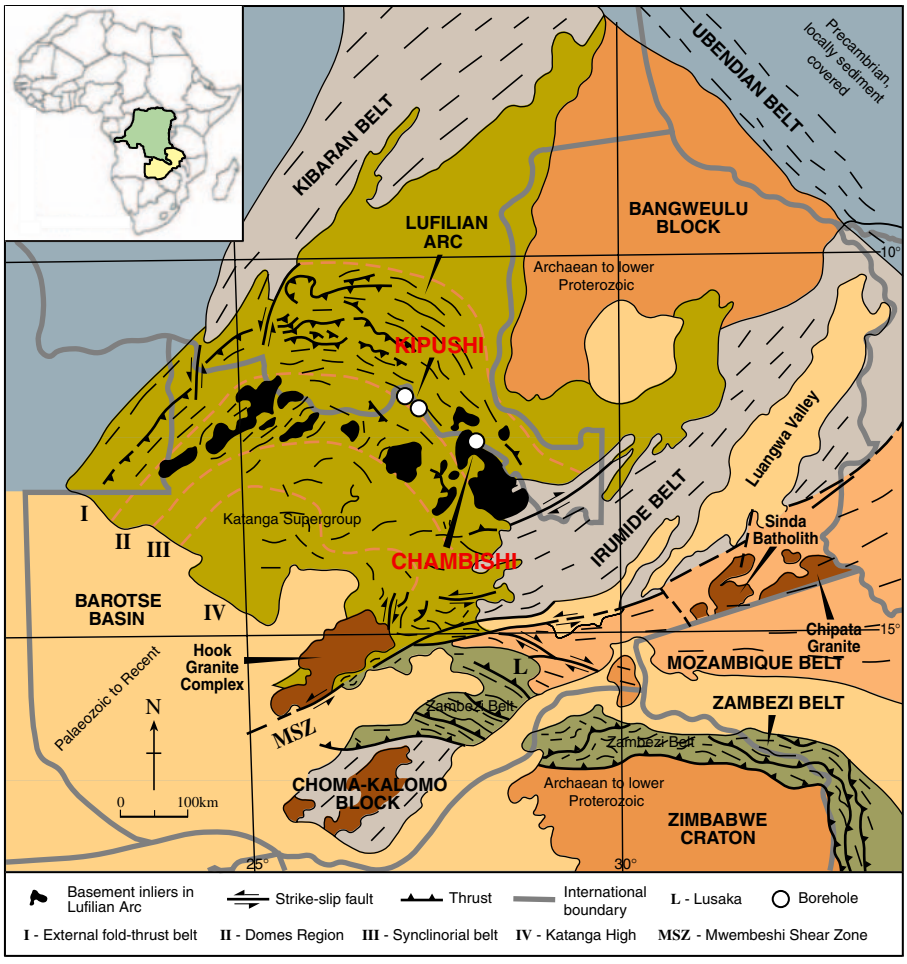

Fig. 1. Geological map of the Lufilian Arc in Congo and Zambia and part of the Zambezi belt, showing location of drill holes [modified after (*29*, *30*)].

Extraterrestrial influx rates (total mass) estimated from ocean sediment studies based on Ir and Os measurements from mid–Pacific Ocean sediment samples range from $78 \times 10^9 \pm 26 \times 10^9$ g year^{-1} in the time interval between 33 and 67 Ma (*14*) to $30 \times 10^9 \pm 15 \times 10^9$ g year^{-1} between 0 and 80 Ma (*22*). This agrees well with other flux estimations from different time intervals from today to 700,000 years ago equal to $\sim 40 \times 10^9$ g year^{-1}, derived from ^{3}He analyses in Pacific, Atlantic, and Indian Ocean sediments (*23*–*25*). If we assume an Ir concentration of 500 ng per g of extraterrestrial material and an Earth surface area of 5.1×10^{18} cm^2, Ir influx rates of 8 ± 3 ng cm^{-2} My^{-1} to 3 ± 1.5 ng cm^{-2} My^{-1} are implied. However, the Ir flux estimated by (*26*), which was calculated from fossil meteorites in Lower Ordovician marine limestones from Kinnekulle, Sweden, at ~ 30 ng cm^{-2} My^{-1}, indicates that accretion rates of extraterrestrial material were an order of magnitude higher during the Early Ordovician than at present.

We made the conservative assumption that all Ir at the KPCCT is extraterrestrial (thus, no background needs to be calculated and removed) and that no extraterrestrial material was deposited by major impact events. Then we integrated the elevated Ir abundances that represent the peak and took a rock density of 2.5 g cm^{-3} into account, to obtain an Ir concentration of 93 ng cm^{-2} at the KPCCT. If the Ir influx rates discussed above are used, a maximum duration of the Marinoan glaciation of 41 ± 20 My and a minimum of 12 ± 4 My are obtained for the Ir flux during 0 to 80 Ma, and a duration of ~ 3 My if we assume the Ir input flux determined for the Early Ordovician. Nevertheless, because there probably was an unusually enhanced flux of extraterrestrial material in the Early Ordovician, we view the normal (0 to 80 Ma) values as the most representative, leading to a most likely duration of the Marinoan glaciation of 12 My.

A problem in Neoproterozoic geology is the lack of reliable radiometric dates. For the Marinoan glaciation, age limits between 610 and 575 Ma are based on assumed relationships of Marinoan-to-Varanger glacial intervals from different locations (*27*), as well as estimations of sedimentation rate for the time between glacial deposition and for the Precambrian/Cambrian boundary (*28*). Recent

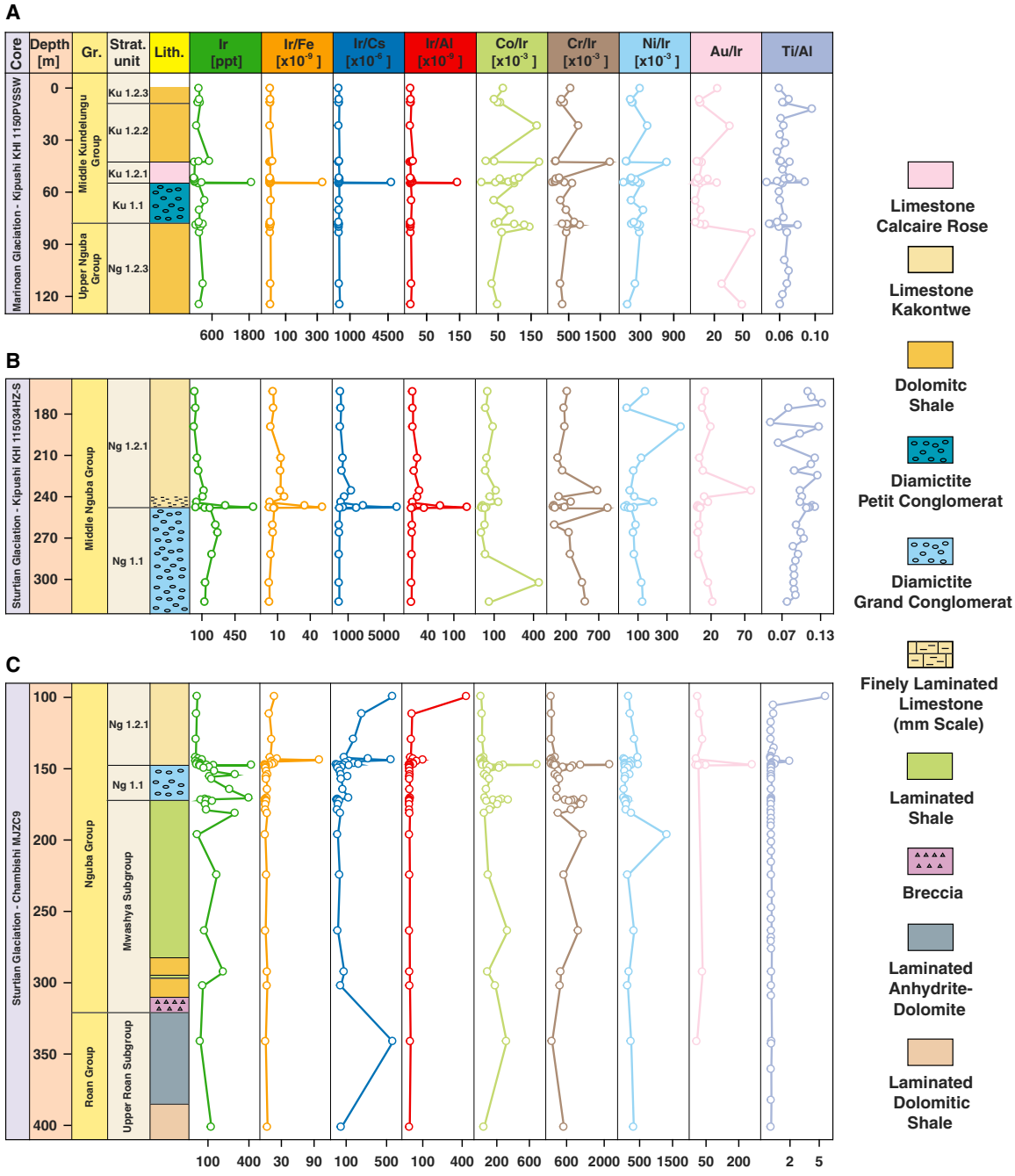

Fig. 2. Stratigraphic columns showing Ir abundances and selected element ratio profiles. (**A**) The middle part of the Kundelungu Group to the top of the Nguba Group, Kipushi, Congo, showing Petit Conglomerat from the Marinoan glaciation overlain by the Calcaire Rose cap carbonates. (**B**) The middle of the Nguba Group, Kipushi, Congo, showing Grand Conglomerat from the Sturtian glaciation overlain by the Calcaire de Kakontwe cap carbonates. (**C**) The Sturtian Grand Conglomerat overlain by the Calcaire de Kakontwe cap carbonates from the middle of the Nguba Group, further to the Mwashya Subgroup at the base of the Nguba Group, and the Upper Roan Subgroup at the top of the Roan Group, Chambishi, Zambia. For tables of elemental concentrations, see (*9*). Analytical uncertainty for Ir is >5 relative % (rel%) for values >500 ppt and 5 to 15 rel% for lower values. Analytical uncertainties for elemental ratios are between 5 and 15 rel%. Ku 1.2.3, dolomitic shale; Ku 1.2.2, dolomitic shale; Ku 1.2.1, Calcaire Rose; Ku 1.1, Petit Conglomerat; Ng 1.2.3, dolomitic shale; Ng 1.2.1, Calcaire de Kakontwe; Ng 1.1, Grand Conglomerat; Strat. Unit, stratigraphic unit; Lith., lithology. The dip of the beds at Kipushi is not vertical but is about 70°. Thus, borehole depths do not represent true stratigraphic thickness.

U-Pb dating indicates that the Marinoan glaciation took place at 635.5 ± 1.2 Ma (*9*). The duration of glaciation in the Neoproterozoic is difficult to quantify because of uncertain age limits and nonuniform, noncontinuous sedimentary records. Previous methods used to estimate the duration of Neoproterozoic glaciation are as follows: magnetic polarity intervals (*8*), calculation of CO_2 amounts needed to overcome a totally frozen Earth (*6*), and thermal subsidence modeling (*2*). The use of Ir anomalies located immediately at the transition from glacial deposits to cap carbonates is an another possible way to estimate the duration of long glacial epochs. The advantage of this method is that samples from only the transition region of an undisturbed stratum are needed, otherwise a permanent ice sheet must have existed during the glaciation. We calculated a minimum duration for the Marinoan glacial epoch of ~3 My, assuming the same Ir flux as obtained for the Lower Ordovician, and a most likely duration of about 12 My, based on the average accretion rate.

For the Sturtian event, no Ir anomaly was found directly at the transition from the Sturtian glacial deposits to cap carbonates in the Chambishi and Kipushi successions, but it was found 9 and 63 cm above, respectively. Furthermore, the extraterrestrial Ir is distributed over a wider interval in the cap carbonate succession. This distribution implies that extraterrestrial material was accumulated in upland areas over thousands or millions of years and was then washed into the sea after deglaciation. Unlike the Marinoan cap carbonates, Sturtian cap carbonates lack a transgressive stage, which implies that seawater did not reach critical oversaturation with respect to carbonate until after the postglacial marine transgression (*3*). Therefore, the extraterrestrial Ir in a global ice cover would have been partly dispersed

before the cap carbonate precipitated. This may explain the lack of a sharp Ir spike at the base of the Sturtian cap carbonate. Alternatively, during the Sturtian glacial epoch, Earth's surface may not have been fully covered with ice on which extraterrestrial material could accumulate for a long time; however, the presence of banded iron formations in and below Sturtian glacials suggests that the ocean was ice-covered at that time (*3*).

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The Brain of LB1, *Homo floresiensis*

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The brain of *Homo floresiensis* was assessed by comparing a virtual endocast from the type specimen (LB1) with endocasts from great apes, *Homo erectus*, *Homo sapiens*, a human pygmy, a human microcephalic, specimen number Sts 5 (*Australopithecus africanus*), and specimen number WT 17000 (*Paranthropus aethiopicus*). Morphometric, allometric, and shape data indicate that LB1 is not a microcephalic or pygmy. LB1's brain/body size ratio scales like that of an australopithecine, but its endocast shape resembles that of *Homo erectus*. LB1 has derived frontal and temporal lobes and a lunate sulcus in a derived position, which are consistent with capabilities for higher cognitive processing.

The type specimen of *Homo floresiensis* (LB1, female) (*1*) has a brain size of ~400 cm^3, which is similar to that of *Australopithecus afarensis* specimen AL 288-1 (Lucy) (*2*), who lived approximately 3.0 million years ago. Yet LB1's species was associated with big-game stone technology, remains of *Stegodon*, and charred animal bones that hint at the use of fire and cooking. Its ancestors also had to cross the sea to reach the Indonesian island of Flores (*3*). Could a tiny hominin with an ape-sized brain really have engaged in such advanced behaviors? Some workers reject the notion that LB1 represents a new species that was closely tied to *H. erectus* (*1*) and suggest instead that it was a pathological human microcephalic (*4*). To help address this debate, we compared three-dimensional computed tomographic (3DCT) reconstructions of the internal braincase (virtual endocasts) that reproduce details of external brain morphology, including sulci, vessels, sinuses, cranial capacity, and shape (*5*–*8*), from LB1, an adult female chimpanzee, an adult female *H. erectus* (specimen ZKD XI), a contemporary woman, and a European microcephalic. To broaden taxonomic comparisons and supplement limited sample size, our analysis also included endocasts of the skulls of specimen Sts 5 (*A. africanus*), specimen KNM-WT 17000 (*Paranthropus aethiopicus*), 10 humans, 10 gorillas, 18 chimpanzees (*9*), an adult female pygmy, and five *H. erectus*.

Our virtual cranial capacity estimate for LB1 is 417 cm^3 (*10*). Virtual endocasts of the microcephalic, modern woman, *H. erectus*, and chimpanzee were scaled to 417 cm^3 to facilitate shape comparisons (Fig. 1 and fig. S2). LB1's shape most resembles that of ZKD XI, which is typical of classic *H. erectus* from China and Java (Trinil) (fig. S3). Both endocasts are noticeably wider caudally than rostrally (Fig. 1A), wider ventrally than dorsally (fig. S2), and relatively long and low in lateral profile (Fig. 1B). However, LB1 lacks the de-

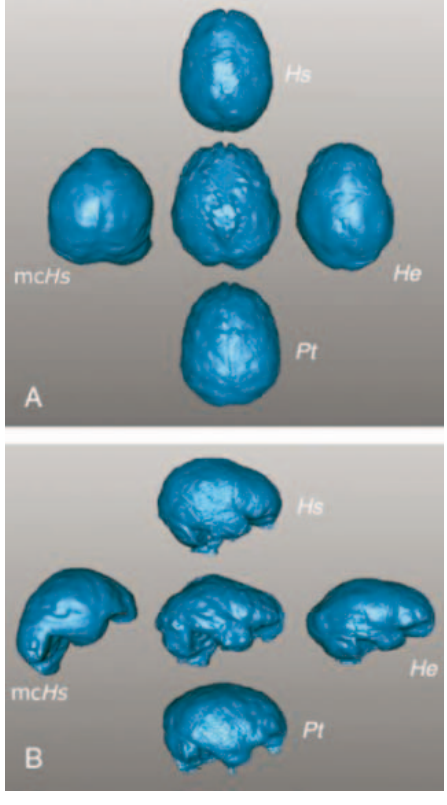

Fig. 1. Comparisons of virtual endocasts of LB1 (center). (**A**) Dorsal views. (**B**) Right lateral views. *Hs*, *H. sapiens*; *Pt*, *Pan troglodytes*; mc*Hs*, a human microcephalic; *He*, *H. erectus*.

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rived occipital expansion over the cerebellum of *H. erectus* (Fig. 1B), and its endocast is relatively wider (more brachycephalic) (Fig. 1A and fig. S3). LB1's endocast least resembles the microcephalic's (Fig. 1 and fig. S2), which has a pointed frontal lobe, compressed occipital lobe, and flattened posterior end, with the caudalmost poles on the cerebellum. Although our sample includes only one microcephalic endocast, its shape conforms to features of its corresponding skull that typify primary microcephaly (microcephalia vera): small cranial vault relative to face, sloping forehead, and pointed vertex (*11*, *12*). The only criterion for secondary microcephaly is an occipitofrontal circumference below –2 SD for age and sex (*11*), but these data are unavailable for LB1's population. Unless a *H. erectus*–like endocast shape is characteristic of an unrecognized form of secondary microcephaly, we reject the hypothesis that LB1 was a pathological microcephalic (*4*).

Length, breadth, height, and frontal breadth measurements were collected from endocasts (Table 1 and table S1) and used to generate six ratios (Table 1). In a principal-components analysis, LB1 groups with *H. erectus* and is separate from *H. sapiens*, Sts 5 (fig. S4), and the pygmy, based on the first principal component (weighted heavily on relative height and the disparity between maximum breadth and frontal breadth), and is separate from *H. erectus* and the microcephalic in the second principal component (weighted heavily on breadth relative to length) (Fig. 2A). LB1 bears little resemblance to the pygmy (fig. S5). Typically, pygmy skulls are over 1000 cm^3 (ours measures 1249 cm^3) and resemble those of neighboring humans in shape (*13*). Unlike LB1, whose brain/body size ratio scales like that of an australopithecine, however, the ratio for pygmies is slightly larger than that found in

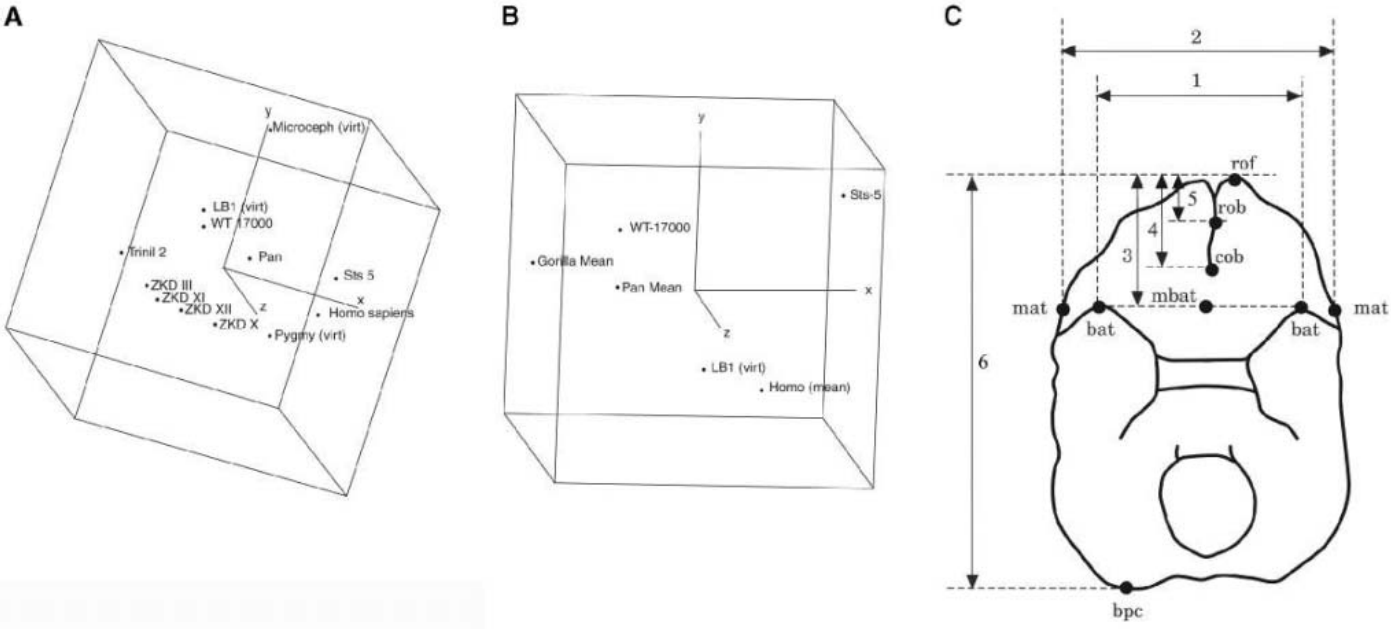

Fig. 2. Plots of principal components and key for basal view measurements. (**A**) Plots of the first three principal components resulting from the analysis of the endocast indices listed in Table 1 [excluding B-FB/H, which was highly correlated with B-FB/L (r = 0.98)]. First, second, and third principal components are aligned along the x, y, and z axes. (**B**) Plots of the first three principal components resulting from the analysis of basal-view endocast indices listed in table S2. (**C**) Key for basal view data analyzed in (B) (*9*). Measurements obtained from basal views were projected onto the horizontal (basal) plane from endocasts. Landmarks: bat, most anterior point on temporal lobe from basal view; mat, most lateral point on endocast at the level of bat in basal plane; mbat, middle of the line connecting the two bats; rof, the most rostral point on the orbital surfaces of the frontal lobes; cob, caudal boundary of olfactory bulbs (cribriform plate) in the midline; rob, rostral boundary of olfactory bulbs in the midline; bcp, most posterior point on the cerebellum in basal view.

Table 1. Endocast measurements (in mm) of length, breadth, height, frontal breadth, and resulting indices.

	Length	Breadth	Height	Frontal breadth	Breadth/ length	Height/ length	Frontal breadth/ length	(Breadth – frontal breadth)/ length	(Breadth – frontal breadth)/ height	Height/ breadth
Pan troglodytes (n = 7)	108.8	88	75.3	72.8	0.81	0.69	0.67	0.14	0.20	0.86
H. sapiens (n = 7)	168.0	128.0	122.0	114.0	0.76	0.73	0.68	0.08	0.11	0.95
KNM-WT 17000*	113.4	92.9	72.5	78.1	0.82	0.64	0.69	0.13	0.20	0.78
Sts 5†	119.1	93.5	86.3	85.6	0.79	0.72	0.72	0.07	0.09	0.92
ZKD III (skull E1)‡	158.6	124.5	99.7	91.4	0.78	0.63	0.58	0.21	0.33	0.80
ZKD X (skull LI)‡	174.6	130.4	114.9	106.7	0.75	0.66	0.61	0.14	0.21	0.88
ZKD XI (skull LII)‡	165.9	127.2	103.7	97.1	0.77	0.63	0.59	0.18	0.29	0.82
ZKD XII (skull LIII)‡	167.4	128	108.5	97.8	0.76	0.65	0.58	0.18	0.28	0.85
Trinil 2§	156.7	126.9	95	92.5	0.81	0.61	0.59	0.22	0.36	0.75
Microcephalic‖	89.1	84.4	66.3	63.7	0.95	0.74	0.71	0.23	0.31	0.79
Pygmy‖	165.7	123.9	116.9	102.6	0.75	0.71	0.62	0.13	0.18	0.94
LB1‖	119.6	102.8	81.4	77.7	0.86	0.68	0.65	0.21	0.31	0.79

**Paranthropus aethiopicus*. †*A. africanus*. ‡*H. erectus* (formerly *Sinanthropus*, China). §*H. erectus* (formerly *Pithecanthropus*, Java). ‖Computer model, virtual endocast.

their nonpygmy neighbors, giving their heads a relatively large appearance (*14*). This is expected because pygmies scale allometrically along ontogenetic curves (*15*), leading to relatively enlarged heads and brains, as is the case for human youngsters relative to adults (*16*) (fig. S1). The laws governing allometric scaling of brain/body ratios are powerful and hold within other species of primates (*17*, *18*). For this reason, and because the morphologies of our endocast samples differ greatly, we do not believe that LB1 represents a human pygmy (*19*).

A second principal-components analysis was performed on measurements from the base of LB1's endocast and compared to similar measurements from 10 gorillas, 18 chimpanzees, 10 *H. sapiens*, KNM-WT 17000 (*Paranthropus aethiopicus*), and Sts 5 (*9*) (Fig. 2, B and C, and tables S2 and S3). The *H. erectus* endocasts were excluded because their bases were missing. The first and second principal-components analyses group LB1 exclusively with *H. sapiens* (Fig. 2B). The first principal component is most heavily weighted on 4/6 and 5/6 (Fig. 2C), which represent the relative projection of the prefrontal cortex rostral to both the anterior and posterior margins of the olfactory bulb. The second principal component is most heavily weighted on 3/6 and (6-3)/6, which represent the relative length of the frontal lobes rostral to the temporal poles and the relative length of the brain caudal to the temporal poles. As in humans, the most anterior sectors of LB1's orbital surfaces are lengthened.

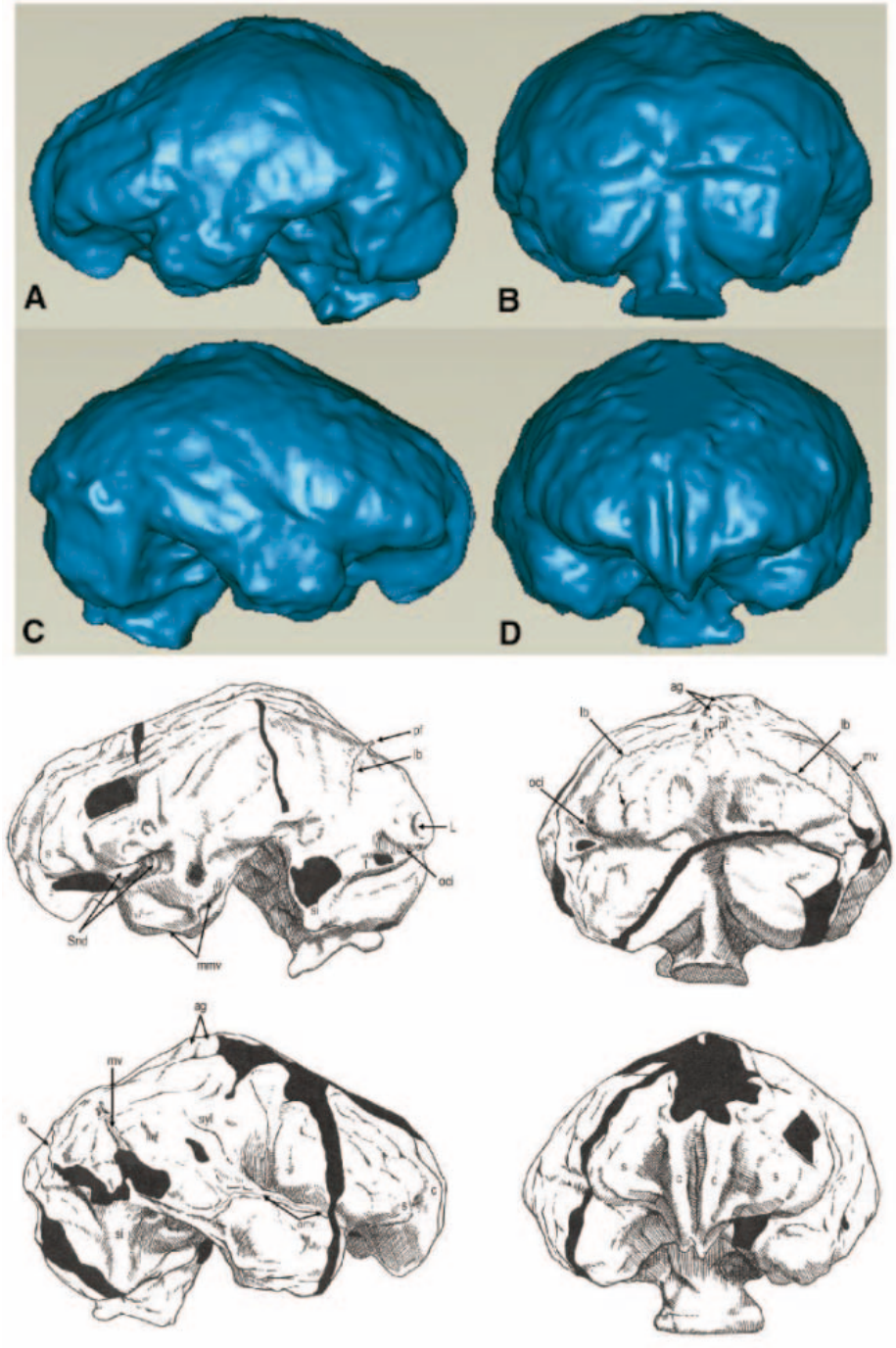

Fig. 3. Virtual endocast of LB1 (top). Views: (**A**), left lateral; (**B**), posterior; (**C**), right lateral; (**D**), frontal. Identifications of features are shown on corresponding sketches (bottom) (damaged areas are blackened) as follows: ag, arachnoid granulations; c, frontal lobe convolutions; lb, lambdoid suture; L, lunate sulcus; mv, meningeal vessels; mmv, middle meningeal vessels; oci, inferior occipital sulcus; omv, orbital meningeal vessels; pf, foramen for parietal emissary vein; s, frontal lobe swelling; si, sigmoid sinus; Snd, Sylvian notch and depression; Syl, Sylvian fissure; t, transverse sinus.

The lambdoid suture is located more rostrally on the left than on the right side of the endocast (Fig. 3). Both the skull and the endocast show a left frontal and right occipital petalia (Fig. 1A) that, in humans, are statistically correlated to some degree with left-handedness (*20*). After entering the middle cranial fossa, small anterior branches of the middle meningeal vessels course rostrally across the ventral surface of the right temporal lobe and across the ventrolateral surface on the left. On the right, a branch from another meningeal vessel enters the middle braincase from the orbital region and courses caudally across the temporal lobe inferior to the Sylvian fissure. Similar orbital contributions are common in apes and have been reported for certain *H. erectus* endocasts by some workers (*21*) but not others, who used a scoring system for modern humans (*22*). Traces of meningeal vessels are also reproduced in the right parietal region, and several arachnoid granulations appear near the vertex on the right. LB1 reproduces somewhat (artifactually) distorted transverse and sigmoid sinuses. A cast of the parietal emissary foramen appears near the medial end of the left lambdoid suture.

The right side of LB1's endocast reproduces part of the Sylvian fissure and numerous small sulci on the lateral temporal and dorsolateral frontal lobes (Fig. 3). The right orbital surface reveals three small sulci that do not extend onto the dorsal surface (the left orbital surface is damaged). In the left occipital region, LB1 reproduces an inferior occipital sulcus and a small crescent-shaped lunate sulcus medial to it and caudal to the lambdoid suture. The position of the lunate sulcus is derived and suggests cortical reorganization in the posterior parietal association cortex as compared with apes (*2*, *23*).

LB1's orbital caps are not delimited rostrally by apelike orbitofrontal sulci that incise the borders and course toward the temporal poles on the orbital surfaces (*23*, *24*). Instead, LB1's gyrification, orientation, and relation-

ship of the lateral prefrontal cortex relative to the temporal poles appear derived. Following Connolly (*23*), we decline to identify rami that border the human pars triangularis (part of Broca's area) on the left, although the general morphology in this region would be consistent with their existence. On the left (and to a lesser extent the right), a distinct Sylvian notch separates the temporal from the frontal lobe and continues caudally as a depression. This region corresponds to a Sylvian crest within the skull of LB1 that, in humans, sometimes occurs in particularly thick skulls and is correlated with Sylvian depressions on endocasts, although the brains are, if anything, more opercularized in the corresponding area (*23*).

The depression for the superior sagittal sinus on LB1's frontal lobes is bordered laterally by large convolutions [which probably contained additional furrows not reproduced on the endocast (*23*)] that curve around the rostral tip of the endocast onto the orbital surface and meet at the foramen caecum. Dimples separate these convolutions laterally from swellings that square off the frontal lobes and give their outline a ruffled appearance in dorsal view (Fig. 1A). Although hints of such contours may be seen in chimpanzee and hominin endocasts such as in the no. 2 specimen from Sterkfontein (*9*), the extent of these expansions in the frontal polar region of LB1 is unusual. This part of the prefrontal cortex in humans and apes consists of Brodmann's area 10, which in humans may be involved in higher cognitive processes such as the undertaking of initiatives and the planning of future activities (*25*). Human frontal lobes are not larger than expected for apes of similar brain volume (*26*), but area 10 is both absolutely and relatively enlarged in *H. sapiens* as compared with apes (*25*). LB1's polar convolutions appear derived compared with those of *H. erectus* and other early hominins. Unlike the frontal lobes, human temporal lobes appear to be somewhat larger than expected for an ape brain of human size (*26–28*); thus, LB1's extremely wide temporal lobes (brachycephaly; fig. S3) may represent another derived feature.

Our data show that LB1's well-convoluted brain could not have been a miniaturized version of the brain of either *H. sapiens* or *H. erectus*. Nevertheless, its similarities with *H. erectus* strongly suggest a phylogenetic connection, although its australopithecine-like brain/body size ratio and morphology of the femur and pelvis (*29*) are not expected in a miniaturized descendant of a larger-bodied *H. erectus* (which, instead, would be expected to scale allometrically along the ontogenetic curve predicted for *H. erectus*) (fig. S1). Although it is possible that *H. floresiensis* represented an endemic island dwarf that, over time, became subject to unusual allometric constraints, an alternative hypothesis is that *H. erectus* and *H. floresiensis* may have shared a common ancestor that was an unknown small-bodied and small-brained hominin (*1*).

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Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 to S3
References

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Vasopressin and Oxytocin Excite Distinct Neuronal Populations in the Central Amygdala

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Vasopressin and oxytocin strongly modulate autonomic fear responses, through mechanisms that are still unclear. We describe how these neuropeptides excite distinct neuronal populations in the central amygdala, which provides the major output of the amygdaloid complex to the autonomic nervous system. We identified these two neuronal populations as part of an inhibitory network, through which vasopressin and oxytocin modulate the integration of excitatory information from the basolateral amygdala and cerebral cortex in opposite manners. Through this network, the expression and endogenous activation of vasopressin and oxytocin receptors may regulate the autonomic expression of fear.

The amygdala plays an important role in anxiety and fear behavior. Fear learning involves its lateral and basolateral parts, where the association between incoming fearful and neutral stimuli leads to potentiation of synaptic transmission. These parts project to the central amygdala (CeA), whose efferents to the hypothalamus and brainstem trigger the autonomic expression of fear (*1*). Selective gating of synaptic transmission through the CeA could therefore modulate the fear response (*2*, *3*). Indeed, recent studies suggest that increased inhibition within the CeA could underlie the anxiolytic effects of benzodiaze-

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pines and alcohol (*4*, *5*) and may also play a role in the extinction of conditioned fear through cortical afferents (*6*, *7*).

The CeA expresses numerous neuropeptides and neuropeptide receptors, including high levels of receptors for vasopressin and oxytocin (*8*, *9*). Activation of vasopressin and oxytocin receptors oppositely affects fear and anxiety-related behaviors. Vasopressin enhances aggressiveness, anxiety, and stress levels and the consolidation of fear memory (*10*–*13*). Oxytocin decreases anxiety and stress and facilitates social encounters, maternal care, and the extinction of conditioned avoidance behavior (*13*–*17*). At the cellular level, however, both neuropeptides increase neuronal excitability in various brain regions, including the CeA (*18*–*20*), which raises the question of whether a local neuronal network could underlie their opposite behavioral effects.

We first determined the distribution of vasopressin and oxytocin receptors in the CeA using autoradiography on horizontal rat brain sections (*21*). Binding of ^{125}I-labeled ligands revealed that expression of oxytocin receptors was restricted to the lateral and capsular division of the CeA (CeL/C) and vasopressin receptors in the medial part (CeM) (Fig. 1A). To determine the physiological effects of activating these receptors, we recorded spontaneous spiking activity extracellularly in acute brain slices of the CeA (*21*). Bath application of the highly specific oxytocin receptor agonist [Thr^4,Gly^7]-oxytocin (TGOT, 0.2 μM, for 30 s) (fig. S2) (*21*) increased spontaneous spike frequencies in 21% of 224 recorded neurons (to 284 ± 26% of the initial frequency) but decreased them in more than 50% (to 19 ± 2%). Both responses were fully reversible and repeatable (fig. S1) and could be blocked by the oxytocin receptor antagonist d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Orn^8,des-Gly-NH$_2^9$]-vasotocin (OTA, 1 μM) [TGOT excitation: 301 ± 16%, $P < 0.05$, and OTA+TGOT: 93 ± 7%, $P > 0.05$, $n = 6$ experiments (*21*); TGOT inhibition: 6 ± 6%, $P < 0.05$, and OTA+TGOT: 110 ± 24%, $P > 0.05$, $n = 5$ experiments; all relative values are expressed as percentages of the control frequency] (Fig. 1, B to D). Subsequent exposure of TGOT-excited cells to the general vasopressin receptor agonist [Arg^8]-vasopressin (AVP, 0.02 to 0.2 μM, for 30 s) was only able to induce small increases in frequency (Fig. 1B), which were probably caused by a cross-reactivity of AVP on oxytocin receptors (fig. S2) (*21*). On the other hand, AVP potently excited more than 50% of TGOT-inhibited neurons (319 ± 9%, 24 out of 47 cells) (Fig. 1, C and D). This latter effect was fully reversible and repeatable (fig. S1) and appeared mediated by V1a receptors: It could be blocked by the V1 receptor antagonist d(CH$_2$)$_5$[Tyr(Me)^2,Arg^8]-vasopressin (TMA, 1 μM) (Fig. 1D) and could be mimicked neither by the V1b receptor agonist [1-deamino-4-cyclohexylalanine]-Arg-vasopressin (d[Cha]AVP, 1 μM) (Fig. 1D) nor by the V2 receptor agonist [deamino-Cys^1,Val^4,D-Arg^8]-vasopressin (dVDAVP, 1 μM) (fig. S3). This was further confirmed by an additional combination of different vasopressin receptor agonists and antagonists (fig. S3). Thus, our findings suggest two groups of TGOT-responsive neurons in the CeA: one that is excited by oxytocin receptor activation and a second that is inhibited by activation of oxytocin receptors but excited by vasopressin V1a receptors.

Earlier morphological studies have shown intense γ-aminobutyric acid (GABA)–positive staining in the CeA and projections from the CeL/C onto the CeM, which are thought to be GABAergic (*8*, *22*). Hypothesizing that these could mediate the inhibitory effects of oxytocin receptor activation, we determined the precise position and projections of TGOT and AVP-excited cells using sharp-electrode intracellular current-clamp recordings. Neurons were held near the spiking threshold (–55 ± 4 mV), and excitation was measured as a rapid increase in spontaneous spike frequency accompanied by small depolarizations (3.4 ± 0.4 mV and 4.6 ± 0.8 mV, respectively; $n = 6$ experiments) that were resistant to tetrodotoxin (TTX, 1 μM) (Fig. 2A). Cells with

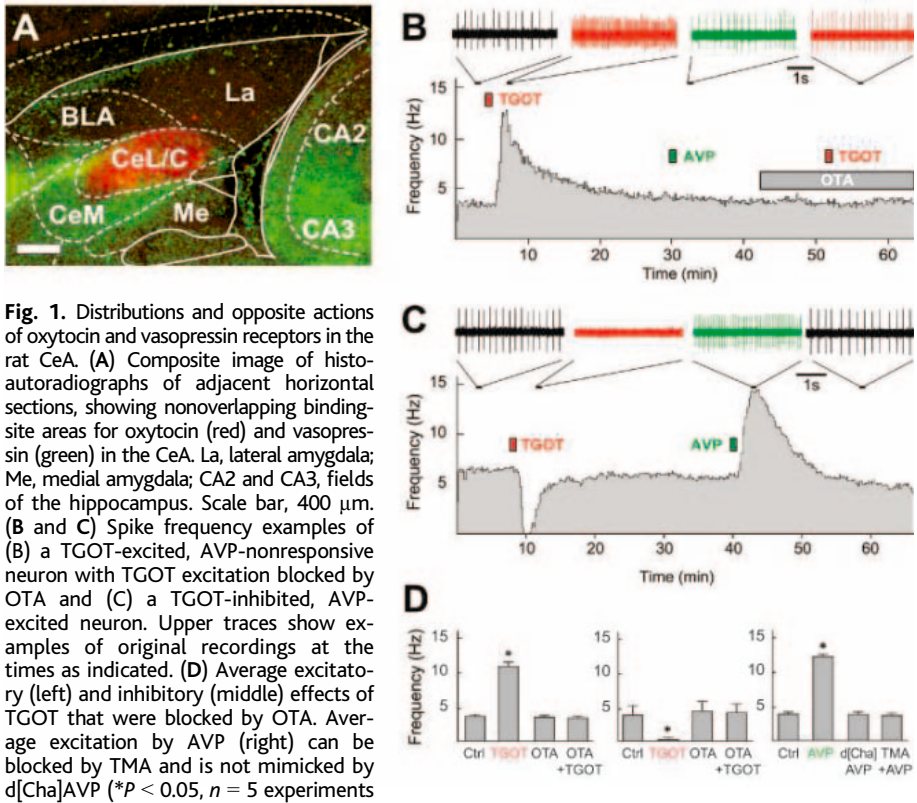

Fig. 1. Distributions and opposite actions of oxytocin and vasopressin receptors in the rat CeA. (**A**) Composite image of histo-autoradiographs of adjacent horizontal sections, showing nonoverlapping binding-site areas for oxytocin (red) and vasopressin (green) in the CeA. La, lateral amygdala; Me, medial amygdala; CA2 and CA3, fields of the hippocampus. Scale bar, 400 μm. (**B** and **C**) Spike frequency examples of (B) a TGOT-excited, AVP-nonresponsive neuron with TGOT excitation blocked by OTA and (C) a TGOT-inhibited, AVP-excited neuron. Upper traces show examples of original recordings at the times as indicated. (**D**) Average excitatory (left) and inhibitory (middle) effects of TGOT that were blocked by OTA. Average excitation by AVP (right) can be blocked by TMA and is not mimicked by d[Cha]AVP (*$P < 0.05$, $n = 5$ experiments for all bars). Ctrl, control.

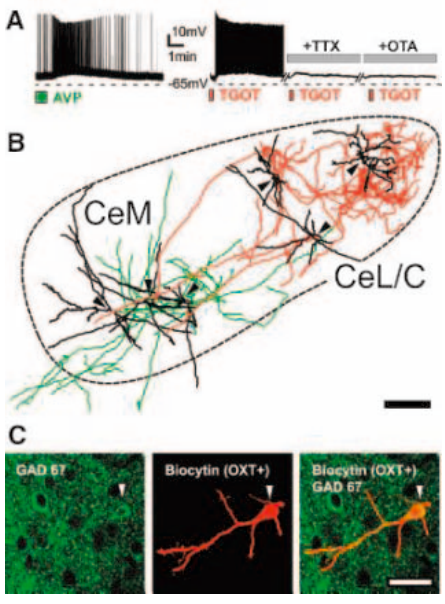

Fig. 2. Intracellular recordings and morphological properties of vasopressin and oxytocin excited neurons in the CeA. (**A**) Intracellular recordings of an AVP-excited neuron in the CeM (left) and a TGOT-excited neuron in the CeL/C (right) with depolarization that persisted in the presence of TTX but was blocked by OTA. (**B**) Morphology and projections of three oxytocin-excited (red axon collaterals) and three vasopressin-excited neurons (green axon collaterals), as revealed by biocytin injections after intracellular recordings (dendrites in black and somata marked by black arrows). Scale bar, 200 μm. (**C**) Neurochemical characterization of an oxytocin-excited neuron (OXT+, white arrow indicates soma) costained for GAD-67 (green) after biocytin injection (red) reveals costaining (yellow). Scale bar, 25 μm.

excitatory responses were injected with biocytin from the intracellular recording pipette (n = 10 experiments). AVP-excited cells were restricted to the CeM and displayed moderately spiny dendrites and medium-sized cell bodies, with axon collaterals that projected in an anteromedial direction outside the CeA. TGOT-excited neurons were found in the CeL, were of the medium-sized spiny type, and contained several local axon collaterals, of which one or more typically projected toward the CeM (Fig. 2B). Confocal microscopy revealed these TGOT-excited cells to be immunopositive for GAD-67 (Fig. 2C), confirming that they were GABAergic.

Would TGOT indeed affect GABAergic transmission in the CeM? We recorded postsynaptic currents in the CeM by the whole-cell voltage-clamp technique. Bath-applied TGOT evoked rapid increases in these currents that were blocked by OTA and completely disappeared in the presence of the GABA(A) receptor antagonist bicuculline (BIC, 20 μM) (Fig. 3A). Amplitudes and rise and decay times were not affected by 0.2 μM nor by 1 μM TGOT (Fig. 3B and table S1). Thus, TGOT appears to specifically enhance GABAergic transmission in the CeM through a rapid and reversible increase of the frequency of the inhibitory postsynaptic currents (IPSCs). Previous studies have shown that oxytocin is able to modulate synaptic transmission by a number of pre- and postsynaptic mechanisms (*18*). We therefore applied TGOT in the presence of TTX (1 μM) to cells that had previously responded to TGOT, but we found no significant effects on the miniature IPSC frequencies, amplitudes (Fig. 3, A and B), rise times, or decay times (table S1), which seems to exclude a postsynaptic effect by TGOT. A presynaptically mediated increase in IPSC frequency could result from an enhanced excitability of the cell body or from an increased release probability from the presynaptic site. We thus focally applied TGOT (1 μM) from a 1-μm patch pipette at the presynaptic site near the recorded neuron in the CeM, but this never caused a change in IPSC frequency (Fig. 3C, position A) (n = 5 experiments). Puffing of TGOT laterally, however, at distant sites in the CeL/C, was able to induce sharp increases in IPSC frequencies at specifically identified locations (Fig. 3C, position B, and table S1) (n = 5 experiments), which were blocked by

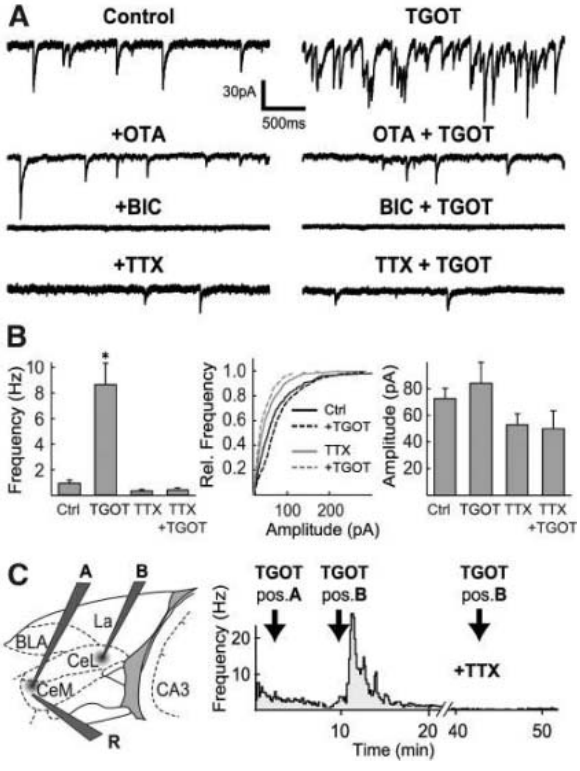

Fig. 3. Local effects of oxytocin on IPSCs in the CeA. (**A**) Examples of IPSC appearances in the presence of various treatments as indicated. (**B**) Average TGOT effects on IPSC frequency and amplitude in the absence and presence of TTX (left, n = 9 experiments); TGOT significantly enhanced mean IPSC frequency (*, $P < 0.01$) but did not affect amplitudes of IPSC or miniature IPSCs ($P > 0.1$, n = 5 experiments, middle and right) (table S1). Rel. Frequency, relative cumulative frequency. (**C**) Effects of local application of TGOT (1 μM) with a patch pipette in the CeM (position A) and in the CeL/C (position B) on IPSC frequency before and after TTX. R indicates the recording electrode.

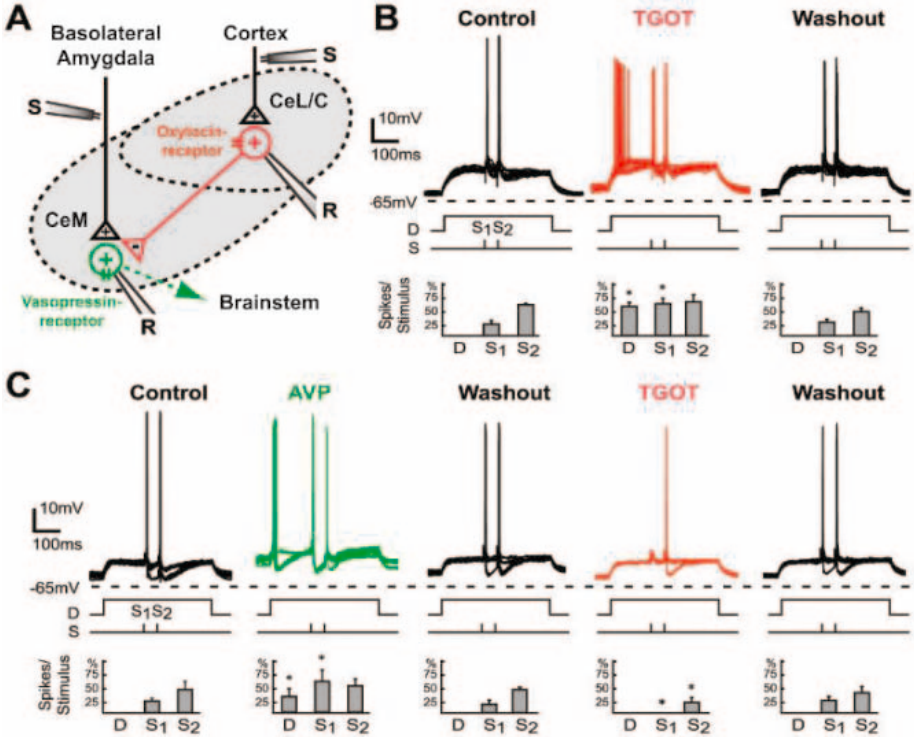

Fig. 4. Effects of oxytocin and vasopressin on the gating of inputs from different afferent pathways in the CeA. (**A**) Simplified model of local circuitry in the CeA, showing processing of different excitatory inputs (+) and GABAergic connections (–) between oxytocin and vasopressin receptor–expressing regions (CeL/C and CeM), stimulation electrode (S), and recording electrode (R). (**B**) Paired stimuli (S1 and S2, 50-ms separation, at 10-s intervals) applied to cortical afferents in the external capsule (*6*) resulted in excitatory potentials in CeL/C neurons under current-clamp. Stimuli were paired with postsynaptic current injections (D) such that the second stimulus (S2) regularly evoked action potentials. TGOT application (1 μM, for 1 min) caused a small depolarization of the membrane potential and increased probability of action potential generation after D, S1, or S2. The traces show superpositions of 15 sweeps, which were averaged per experiment in order to calculate the percentages of spikes evoked by each stimulus as indicated by the bar charts below (*, $P < 0.05$, n = 5 experiments). (**C**) Neurons in the CeM were stimulated through their afferents in the basolateral and basomedial nuclei (*22*). AVP (0.2 μM, for 1 min) caused similar effects as TGOT in the CeL, whereas subsequent administration of TGOT (1 μM, for 1 min) led instead to decreases in responses to S1 and S2 (*, $P < 0.05$, n = 5 experiments).

subsequent application of TTX. These findings indicate that the inhibitory effects of TGOT are caused by an enhanced excitability of neurons in the CeL/C that leads to an increase of GABA release in the CeM.

Changes in the excitability of neurons in different subnuclei may be relevant to the behavioral function of the CeA, because they can modulate the integration of its distinct inputs (*1*). The CeL/C receives projections from cortical and subcortical areas (*6*, *8*) and projects to the CeM, which also receives direct input from the basolateral amygdala (BLA) (Fig. 4A) (*22*). We indeed found that during stimulation of the excitatory afferents to the CeL/C, TGOT could enhance the probability of evoking postsynaptic action potentials (Fig. 4B). During stimulation of the excitatory afferents to the CeM, however, TGOT decreased the probability of evoking postsynaptic action potentials, but AVP increased it (Fig. 4C).

These findings reveal two major points. First, vasopressin and oxytocin modulate activity in CeM neurons in opposite ways through the activation of distinct elements of an inhibitory network (Fig. 4A). Second, through the activation of these distinct elements, vasopressin and oxytocin can differently affect the integration of distinct afferents to the CeA into a common output to the autonomic nervous system, thus providing a neurophysiological mechanism for their opposite effects on anxiety and fear behavior. As we have previously found a comparable distribution of oxytocin and vasopressin receptors throughout the central extended amygdala (*9*), this mechanism may also apply to regions that include the bed nucleus of the stria terminalis and parts of the nucleus accumbens. These latter structures are known to be involved in the control of anxiety, stress, motivation, and addiction (*23*) and are possibly regulated by vasopressin and oxytocin in a similar manner.

The results of this study suggest that the endogenous balance between oxytocin and vasopressin receptor expression and activation may set distinct, individually tuned levels for the activation of the autonomic fear response. The levels of these neuropeptides in the extracellular fluid of the CeA are increased during stress (*12*, *24*), possibly through release from local vasopressinergic and oxytocinergic fibers (*25*, *26*). Furthermore, variations in levels of receptor expression and injections of specific antagonists have been directly correlated with changes in anxiety and fear (*12*, *16*, *17*, *24*, *27*, *28*). Together, these findings confirm the physiological and behavioral relevance of the proposed mechanism. Anxiety and fear can directly affect parental care, thereby modulating the expression of oxytocin and vasopressin receptors in offspring and establishing anxiety and fear traits that can be carried over several generations (*16*, *27*). The elucidation of the opposite, modulatory mechanism of these two peptides in the CeA provides a solid rationale for the development of new, individually tailored treatments, working in concert with the more traditional GABAergic agonists (*4*, *5*). Indeed, the vasopressin receptor could be a pharmacological target for the treatment of stress and anxiety-related disorders (*10*, *11*).

Several recent lines of evidence suggest that fear extinction inhibits the expression of the conditioned reaction rather than erasing the memory (*3*). This inhibition is thought to be mediated by cortical afferents to the amygdala, originating in the medial prefrontal cortex (*7*). Our findings provide evidence for a functional link between cortical input in the CeL/C and inhibition of output from the CeA. The oxytocinergic modulation of the cortical input and the vasopressinergic effects on input from the BLA could implicate additional, opposing roles for these neuropeptides in fear extinction.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5719/245/DC1
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Figs. S1 to S3
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Dependence of Self-Tolerance on TRAF6-Directed Development of Thymic Stroma

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The microenvironments of the thymus are generated by thymic epithelial cells (TECs) and are essential for inducing immune self-tolerance or developing T cells. However, the molecular mechanisms that underlie the differentiation of TECs and thymic compartmentalization are not fully understood. Here we show that deficiency in the tumor necrosis factor receptor–associated factor (TRAF) 6 results in disorganized distribution of medullary TECs (mTECs) and the absence of mature mTECs. Engraftment of thymic stroma of *TRAF6*$^{-/-}$ embryos into athymic nude mice induced autoimmunity. Thus, TRAF6 directs the development of thymic stroma and represents a critical point of regulation for self-tolerance and autoimmunity.

Thymic epithelial cells (TECs) establish spatially distinct microenvironments that are essential for generating a T cell repertoire. Cortical TECs (cTECs) are involved in selecting thymocytes that are capable of recognizing self–major histocompatibility complex (*1*),

whereas medullary TECs (mTECs) play a crucial role in self-tolerance by eliminating self-reactive T cells (*2*). However, the molecular mechanisms underlying the differentiation and organization of TECs are not fully understood.

Tumor necrosis factor receptor (TNFR)–associated factor 6 (TRAF6) transduces signals from members of the TNFR superfamily and the Toll/interleukin-1R family, leading to the activation of transcription factors such as nuclear factor κB (NF-κB) and activating protein 1 (AP1) (*3*). We previously reported thymic atrophy in *TRAF6*$^{-/-}$ mice (*4*), which led us to hypothesize that TRAF6 might be involved in thymic organogenesis.

To explore this in more detail, formalin-fixed sections of thymi from 14-day-old mice were analyzed by hematoxylin and eosin (H&E) staining. The size of the medulla was reduced, and the corticomedullary junction was obscure in *TRAF6*$^{-/-}$ mice (Fig. 1A). Adjacent thymic sections were then used for immunohistochemistry. We visualized mTECs with an antibody that recognizes keratin-5, an mTEC marker, and *Ulex europaeus* agglutinin-1 (UEA-1), a lectin that binds mature mTECs (*5*–*7*). Keratin-5^{+} cells and UEA-1^{+} cells were clustered in the medulla in the wild-type thymus, whereas keratin-5^{+} cells were dispersed and UEA-1^{+} cells were absent in the *TRAF6*$^{-/-}$ thymus (Fig. 1B). CD11c^{+} dendritic cells (DCs) and Ly51^{+} cTECs were distributed normally in the *TRAF6*$^{-/-}$ medulla and cortex, respectively (Fig. 1B). Investigation of thymi at various embryonic stages revealed that UEA-1^{+} cells, which appear in the wild-type thymus by embryonic day 16 (E16), were not observed in the *TRAF6*$^{-/-}$ thymus (Fig. 1C and fig. S1). These data revealed abnormal distribution of mTECs and their impaired maturation into UEA-1^{+} cells in the *TRAF6*$^{-/-}$ thymus.

The *aire* gene is preferentially expressed in mTECs (*5*), and Aire protein promotes the ectopic expression of peripheral tissue-specific antigens (TSAs), thereby establishing central tolerance to TSAs (*8*, *9*). Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) analyses revealed that expression of *aire* and TSAs are significantly reduced in the 14-day-old *TRAF6*$^{-/-}$ thymus (Fig. 1D).

The altered thymic organization and reduced *aire* expression led us to hypothesize that *TRAF6*$^{-/-}$ mice may possess an autoimmune phenotype, which was supported by our observations of inflammatory infiltrates in the lung, liver, pancreas, and kidney of *TRAF6*$^{-/-}$ mice and augmentation of activated CD4^{+} T cells in the *TRAF6*$^{-/-}$ lung (fig. S2). Analysis of thymopoiesis in 14-day-old mice suggested that the number of regulatory T cells (T_{reg}s) was dramatically reduced as determined by the loss of CD25^{+} T cells (Fig. 1E), whereas maturation of T cells as judged by CD69 and CD24 expression and generation of natural killer T (NKT) cells were not significantly affected in the *TRAF6*$^{-/-}$ thymus (fig. S3).

To determine whether the autoimmune-like phenotypes of *TRAF6*$^{-/-}$ mice were related to the altered thymic stroma, fetal thymi isolated from E14 *TRAF6*$^{-/-}$ and control embryos on a BALB/c background were depleted of hematopoietic cells by incubation in 2′-deoxyguanosine (2-DG) and then grafted under the renal capsule of BALB/c$^{nu/nu}$ mice. Eight weeks after grafting, normal generation of thymocytes (Fig. 2A) and distribution of mature T cells in spleen and lymph nodes (fig. S4) were observed in recipients grafted with *TRAF6*$^{-/-}$ thymus [knock-out/nude (KO/nu)] mice as well as in recipients grafted with control thymus [wild-type/nude (WT/nu)]

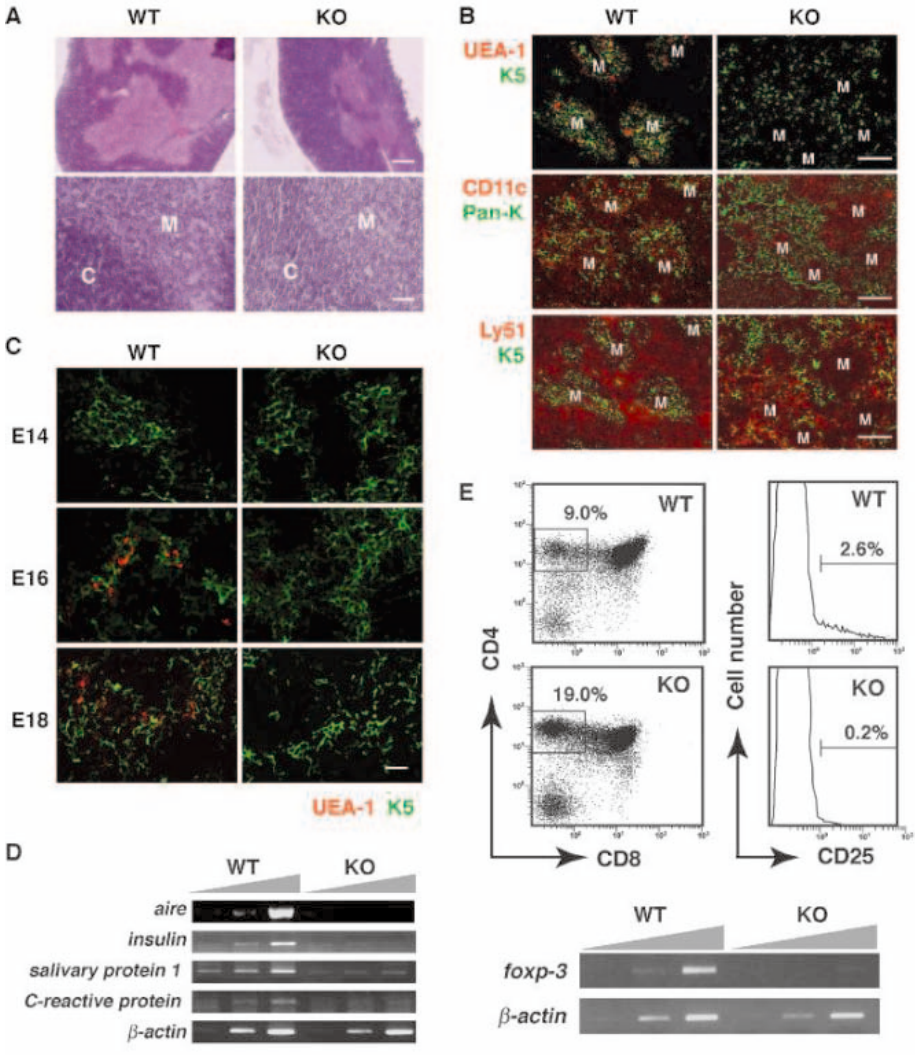

Fig. 1. TRAF6 is necessary for the organization and maturation of mTECs. (**A**) H&E staining of formalin-fixed thymic sections. Scale bars, 200 μm (top) and 20 μm (bottom). M, medulla; C, cortex. (**B**) Development of mTECs. Adjacent thymic sections are stained with the combination of UEA-1 and antibody to keratin-5 (K5), antibodies to CD11c and pan-keratin (Pan-K), and antibodies to Ly51 and keratin-5. UEA-1, CD11c, and Ly51 are stained red. Keratin-5 and pan-keratin are stained green. M, medulla. Scale bars, 200 μm. (**C**) Development of mTECs during embryogenesis. Thymic sections of embryos are stained with both UEA-1 (red) and antibody to keratin-5 (green). Scale bars, 40 μm. (**D**) Semiquantitative RT-PCR (10-fold serial dilutions) of *aire* and peripheral TSAs. (**E**) Reduction of regulatory T cells in the *TRAF6*$^{-/-}$ thymus. Expression of CD25 on CD4 single positive cells enclosed on the left is shown as histograms on the right (upper). Semiquantitative RT-PCR (10-fold serial dilutions) of foxp-3 is shown at the bottom. 14-day-old mice were used in (A), (B), (D), and (E).

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mice. Histochemical analysis of adjacent sections from the grafted *TRAF6*$^{-/-}$ thymus revealed indistinct corticomedullary junctions, the absence of UEA-1^{+} cells, and scattered keratin-5^{+} cells, in contrast to the normal architecture of the grafted control thymus (Fig. 2B). CD11c^{+} DCs were distributed normally in the medulla in both the grafted *TRAF6*$^{-/-}$ and control thymi (Fig. 2B). These data suggest that generation of UEA-1^{+} mTECs and the clustering of keratin-5^{+} mTECs in the medulla require TRAF6 expression in thymic stroma. KO/nu mice had inflammatory infiltrates in the lung, liver, pancreas, and kidney (Fig. 2C), similar to those in *TRAF6*$^{-/-}$ mice (fig. S2A). KO/nu mice displayed significant splenomegaly (fig. S5), similar to *TRAF6*$^{-/-}$ mice (*4*), and serum immunoglobulin G (IgG) levels of the KO/nu mice were approximately two times higher than those of the WT/nu mice (Fig. 2D). Immunostaining of *RAG-2*$^{-/-}$ tissue sections with serum from the recipient nude mice revealed autoantibodies against whole liver, islets of Langerhans of the pancreas, and blood vessel walls of the lung in KO/nu but not in WT/nu mice (Fig. 2E). These findings indicate that the *TRAF6*$^{-/-}$ altered thymic stroma was sufficient to induce autoimmunity.

Medullary thymic atrophy, lack of UEA-1^{+} mTECs, suppression of *aire* expression, multiorgan inflammation, and impaired negative selection were reported as abnormalities of mice deficient in RelB, a member of the NF-κB family (*10*–*14*), suggesting a possible functional linkage between TRAF6 and RelB in thymic organogenesis. RelB expression in the thymus was up-regulated between E13 and E14, whereas TRAF6 expression reached a plateau at E13 (Fig. 3A). RelB and its transcripts were not detected in 2-DG–treated *TRAF6*$^{-/-}$ fetal thymic stroma (Fig. 3B), indicating that RelB expression is regulated at the mRNA level. RelB is expressed mainly in the thymic medulla in wild-type mice and in the grafted wild-type thymus, whereas RelB expression is significantly reduced in the thymus of *TRAF6*$^{-/-}$ mice and grafted *TRAF6*$^{-/-}$ thymus (Fig. 3C). To investigate the TRAF6-RelB linkage, four independent mTEC lines were established from thymi of wild-type and *TRAF6*$^{-/-}$ E14 embryos (*15*) (fig. S6A). RelB expression in *TRAF6*$^{-/-}$ mTEC lines (KO1 and KO2) was much lower than that in wild-type mTEC lines (WT1 and WT2) (fig. S6B) as observed in vivo (Fig. 3B). Introduction of TRAF6, but not green fluorescent protein (GFP) alone, into KO1 cells enhanced RelB expression (Fig. 3, D and E). These results strongly suggest intracellular linkage between the TRAF6 signal and induced transcription of the *relB* gene, an inference supported by the previous finding that *relB* expression was transcriptionally induced by RelA through the NF-κB–binding site in the *relB* promoter

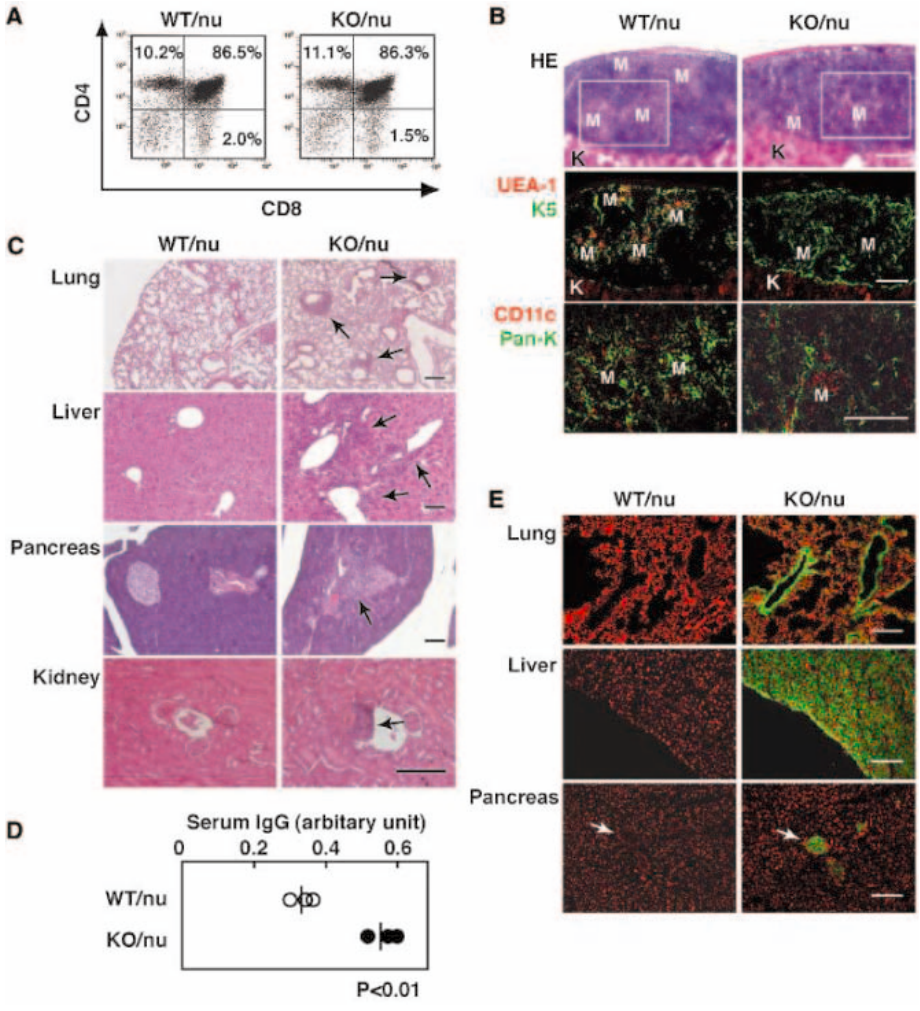

Fig. 2. Autoimmunity induced by grafting *TRAF6*$^{-/-}$ fetal thymic stroma into athymic nude mice. (**A**) Fluorescence-activated cell sorting (FACS) analysis of thymocytes in grafted wild-type and *TRAF6*$^{-/-}$ thymi. (**B**) Abnormal organization of mTECs in grafted *TRAF6*$^{-/-}$ thymus. Adjacent sections of the grafted wild-type or *TRAF6*$^{-/-}$ thymus were stained with H&E, UEA-1 (red), and antibody to keratin-5 (green) and with antibodies to CD11c (red) and pan-keratin (green). M, medulla; K, kidney. Areas surrounded by rectangles in the top panels are magnified in the bottom panels. Scale bars, 200 μm. (**C**) H&E staining of formalin-fixed sections of the lung, liver, pancreas, and kidney of nude mice grafted with wild-type or *TRAF6*$^{-/-}$ fetal thymic stroma. Arrows indicate infiltrates. Scale bars, 200 μm. (**D**) Increased serum IgG levels in recipient mice grafted with *TRAF6*$^{-/-}$ thymus. (**E**) Generation of autoantibodies in recipient mice grafted with *TRAF6*$^{-/-}$ thymus. Serums of nude mice grafted with wild-type or *TRAF6*$^{-/-}$ fetal thymic stroma were tested for the presence of autoantibodies (green) against lung, liver, and pancreas from *RAG-2*$^{-/-}$ mice. Nuclei are stained red with propidium iodide. Arrows indicate islets of Langerhans. Scale bars, 200 μm. All experiments in Fig. 2 were performed 8 weeks after engraftment.

(*16*). Two putative AP1 binding sites present in the *relB* promoter (*16*) also suggest the involvement of TRAF6-induced activation of the mitogen-activated protein kinase family in *relB* induction.

RelB forms heterodimers with p50, a processed product of p105, or p52, a processed product of p100 (*17*). The processing of p105 is constitutive, whereas processing of p100 is triggered by IκB kinase α (IKKα)–mediated phosphorylation of p100. The NF-κB–inducing kinase (NIK) binds and activates IKKα in a signal-dependent manner (*18*). The alymphoplasia (*aly*) strain of mice carries a natural mutation in the NIK gene that renders the NIK protein unable to bind IKKα (*19*) and displays thymus abnormalities similar to those of *TRAF6*$^{-/-}$ and *relB*$^{-/-}$ mice (*20*). Furthermore, thymic stroma from *aly* mice, in which RelB induction and p100 processing are impaired, induce autoimmunity in athymic nude mice (*20*). Although p52$^{-/-}$ mice showed no major defect in the thymus (*21*), mice lacking p100 but still containing a functional p52 protein showed abnormal thymic organogenesis (*22*). Given that p100 has an IκB-like activity (*23*), these results suggest that optimum processing of p100 to p52, which might

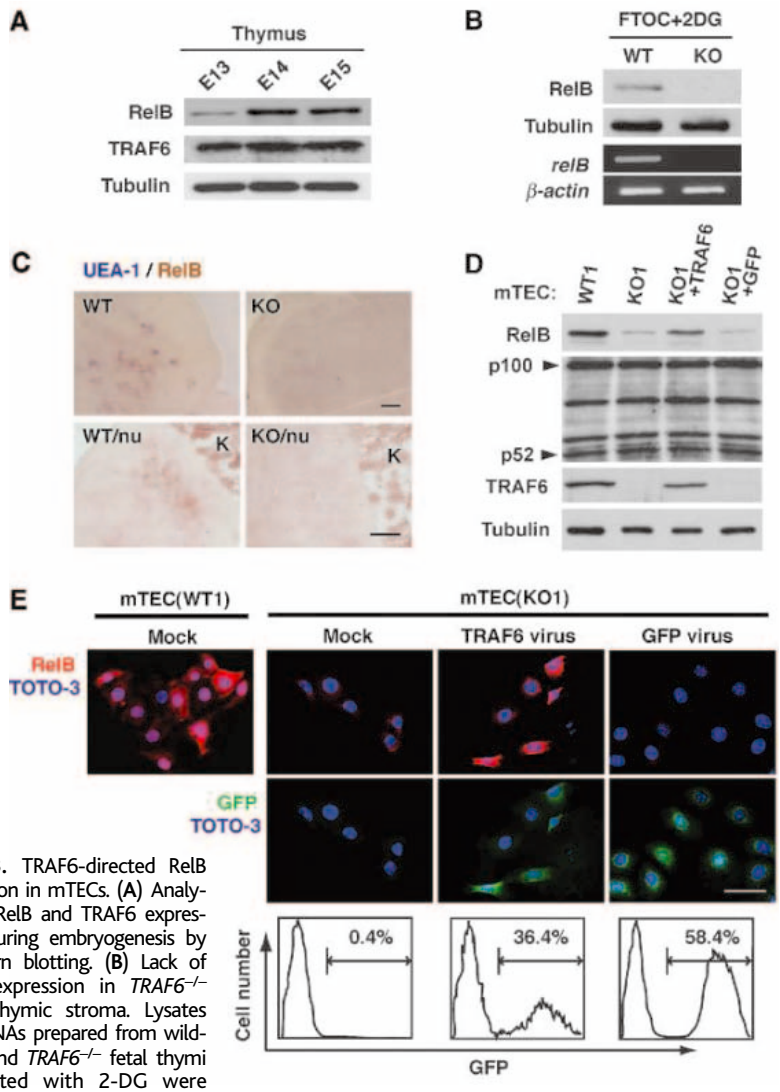

Fig. 3. TRAF6-directed RelB induction in mTECs. (**A**) Analysis of RelB and TRAF6 expression during embryogenesis by Western blotting. (**B**) Lack of RelB expression in *TRAF6*$^{-/-}$ fetal thymic stroma. Lysates and RNAs prepared from wild-type and *TRAF6*$^{-/-}$ fetal thymi incubated with 2-DG were analyzed by Western blotting and RT-PCR. (**C**) Reduced expression of RelB in the medulla of *TRAF6*$^{-/-}$ thymus. Thymi of 14-day-old wild-type and *TRAF6*$^{-/-}$ mice (top) and wild-type and *TRAF6*$^{-/-}$ thymus grafted on nude mice (bottom) were stained with UEA-1 (top, purple) and antibody to RelB (all panels, brown). K, kidney. Scale bars, 200 μm. (**D**) TRAF6 is required for RelB induction in the mTEC lines. The WT1 line was mock infected. The KO1 line was mock infected or infected with retrovirus expressing both TRAF6 and GFP (TRAF6) or GFP alone (GFP). Lysates were analyzed by Western blotting. (**E**) Intracellular linkage of the TRAF6 signal and RelB induction. The WT1 and KO1 lines were infected as described in (D). Four days after infection, a portion of the cells was stained for RelB (red), GFP (green), and nuclei (TOTO-3, blue), and another portion was analyzed for GFP expression by FACS. Scale bars, 50 μm.

lead to optimum expression of some NF-κB–inducible genes, is critical for differentiation of mature mTECs. The ratio of p52 to p100 was not affected by *TRAF6* deficiency (Fig. 3D). Therefore, at least two critical NF-κB–related events could be essential for the initial stage of mTEC differentiation: the induction of RelB expression, which requires both TRAF6 and NIK, and optimum processing of p100 to p52, which requires NIK. It remains unclear whether TRAF6 and NIK participate in the same or different signaling cascades to induce RelB expression. Although the lymphotoxin-β receptor (LTβR) is involved in the development of UEA-1^{+} mTECs (*24*), TRAF6 may not be involved in LTβR signaling, because TRAF6 does not bind the cytoplasmic tail of LTβR (*25*) and *TRAF6* deficiency affects neither LTβR-induced NF-κB activation (fig. S7) nor the formation of Peyer's patch anlagen (*26*).

The inflammatory phenotype of KO/nu mice was milder than that of *TRAF6*$^{-/-}$ mice (Fig. 2C and fig. S2A). (The levels of infiltrate were lower in KO/nu mice than in *TRAF6*$^{-/-}$ mice.) It was recently reported that *TRAF6* deficiency in hematopoietic cells induces T_H2-polarized disease (*27*). Thus, the severe inflammatory phenotype in *TRAF6*$^{-/-}$ mice may result from dysfunctions in both hematopoietic cells and thymic stroma, a possibility that would also explain why an autoimmune phenotype develops earlier in *TRAF6*$^{-/-}$ mice than in *aire*$^{-/-}$ (*8*) and *ltβr*$^{-/-}$ mice (*28*).

Autoimmunity induced by *TRAF6*$^{-/-}$ thymic stroma may be due to a defect in *aire* gene expression or a defect in the production of T_{reg}s. Reduced T_{reg} production is also observed in *aly* (*20*) mice, whereas the number of T_{reg}s is normal in *aire*$^{-/-}$ mice (*8*, *9*). These results suggest that the normal development of T_{reg}s requires thymic microenvironments whose formation is directed by both TRAF6 and NIK-mediated signals. Further studies will clarify how TRAF6-mediated RelB induction leads to *aire* expression and production of T_{reg}s. Our finding that TRAF6 regulates the formation of thymic microenvironments defines a critical step in the development of self-tolerance and may illuminate novel approaches to prevent and treat autoimmune diseases.

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Antigen Recognition Determinants of γδ T Cell Receptors

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The molecular basis of γδ T cell receptor (TCR) recognition is poorly understood. Here, we analyze the TCR sequences of a natural γδ T cell population specific for the major histocompatibility complex class Ib molecule T22. We find that T22 recognition correlates strongly with a somatically recombined TCRδ complementarity-determining region 3 (CDR3) motif derived from germ line–encoded residues. Sequence diversity around these residues modulates TCR ligand-binding affinities, whereas V gene usage correlates mainly with tissue origin. These results show how an antigen-specific γδ TCR repertoire can be generated at a high frequency and suggest that γδ T cells recognize a limited number of antigens.

The γδ and αβ T cells contribute to host immune defense in distinct ways. Whereas αβ T cells are essential in pathogen clearance, γδ T cells have been implicated in the regulation of the immune response (*1*). Although it is clear that γδ T cells can recognize antigens directly without antigen processing and presentation requirements (*2*), it is unclear what the majority of γδ T cell ligands are and how they are recognized. This has made it difficult to define the precise function of γδ T cells. Previously, we found that the closely related major histocompatibility complex (MHC) class Ib molecules T10 and T22 (94% amino acid identity) are induced on activated cells and are ligands for a sizable population (~0.1% to 2%) of γδ T cells in unimmunized mice (*3*). This is potentially an important γδ T cell-ligand pair that could help to regulate immune cells. To understand how this antigen-specific repertoire is generated, particularly the high initial frequency of these cells, we used a T22 tetrameric staining reagent to identify and isolate T22-specific γδ T cells and determined their TCR sequences.

Most splenic γδT cells express Vγ1 and Vγ4, whereas Vγ7-expressing γδ T cells are more prevalent in the intestinal intraepithelial lymphocyte (IEL) compartment (*4*–*6*). This bias in Vγ usage has led to the suggestion that Vγ-encoded residues enable these T cells to respond to antigens unique to their resident tissues (*1*, *7*). Because T22-specific γδ T cells are present in both the spleen and IEL compartments, we first tested whether T22 specificity correlates with V gene usage (*8*). We found that multiple Vγs and Vδs are associated with T22-specific γδ T cells from these two tissues; however, the majority of T22 tetramer-positive cells express Vγ1 and Vγ4 in the spleen, whereas a sizable population of these cells express Vγ7 in the IEL compartment (Fig. 1A and table S1 and S3). This result indicates that Vγ usage is more reflective of the tissue origin than of the antigen specificity for this ligand.

We then compared the TCR sequences of individual T22 tetramer-positive and -negative cells (*8*). Although no conserved sequences in T22-specific TCRγ chains can be identified (tables S1 to S4 and fig. S1), we found that ~90% of the tetramer-positive IELs and ~40% to 60% of the splenic tetramer-positive TCRs contained a prominent CDR3δ sequence motif (Fig. 2A). This motif is also present in the T22-specific G8 and KN6 TCRs (*9*, *10*) but is absent from tetramer-negative splenic cells and more than 98% of the tetramer-negative IELs (tables S1 and S3). This motif consists of a tryptophan (W) encoded by the Vδ or Dδ1 gene segments and the sequence serine–glutamic acid–glycine–tyrosine–glutamic acid (SEGYE), followed by a P nucleotide–encoded leucine (L). Other than the motif, the CDR3δ sequences are diverse, encoded by various Vδs, N and P nucleotides, and Dδ1 in different lengths and reading frames. It is interesting that Vδ6A is the only Vδ to encode a tryptophan residue in the CDR3δ and is overrepresented in T22-specific γδ TCRs (Fig. 1B). Additionally, the CDR3δ length distribution is narrower and longer than that of γδ TCRs in general (Fig. 2, B and C).

To test whether TCRs derived from T22 tetramer-positive cells confer T22 binding specificity, we expressed several of these TCRs in the TCRβ-deficient Jurkat T cell line J.RT3-T3.5, which lacks endogenous surface TCR expression (*8*, *11*). We found that cells expressing TCRs that have the W-(S)EGYEL motif could bind T22 tetramer, whereas those that lack this motif could not (Fig. 3 and fig.

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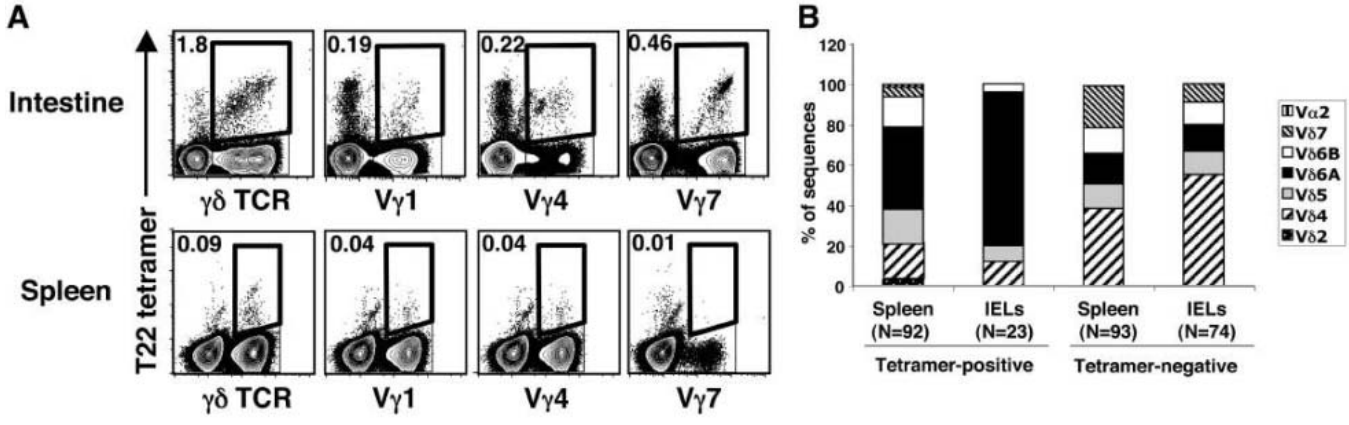

Fig. 1. (**A**) Staining of T22 tetramer with antibodies against Vγ1, Vγ4, and Vγ7 on splenic γδ T cells and IELs (antibodies to Vγ2, Vγ3, and Vγ6 are not available). Number within the plot indicates the percentage of total γδ T cells that are T22 tetramer-positive and Vγ-positive as shown in the box. (**B**) Relative frequency of Vδ usage of T22 tetramer-positive TCR sequences (tables S1 to S4) (*N* is total number of in-frame rearrangements analyzed).

S2). Thus, the higher rate of splenic tetramer-positive T cells without the TCRδ motif may be due to a higher false-positive rate in identifying these cells. This may be caused by the experimental limit associated with fluorescence-activated cell sorting (FACS), especially for low tetramer binders. (T22 tetramer stains IELs at a higher intensity than splenic cells.) Indeed, more recent experiments with a slightly more stringent FACS gating showed that ~70% of the splenic tetramer-positive cells have the TCRδ motif (*12*). Regardless, although both KN6 and 93A10 TCRs use a Vγ4-Vδ5 gene combination, only KN6 contains the W-(S)EGYEL CDR3δ motif and is T22-specific. G8 (Vγ4-Vα11.3), KN6 (Vγ4-Vδ5), as well as 93B7, 93D11, and 917B7 (Vγ1-Vδ6A), all bind T22 but use three different Vγ-Vδ pairs. This indicates that the W-(S)EGYEL CDR3δ motif correlates much better than V gene usage with antigen recognition. Consistent with this is the structural analysis of the G8-T22 complex showing that the residues W and GYEL in the G8 TCR CDR3δ are the principal T22 contact residues (*13*).

To test whether variability in the sequences surrounding the W-(S)EGYEL motif influences ligand binding, we compared the T22 binding characteristics of cells expressing similar levels of the 93B7, 93D11, and 917B7 TCRs, which differ only in those residues. As shown in Fig. 3, these TCRs exhibit significant differences in the half-life ($t_{1/2}$) and affinity (K_D) of T22 tetramer binding. Thus, sequence variations around this motif can modulate the affinity and the kinetics of ligand binding.

These results indicate that, for T22 specificity, a CDR3δ sequence generated by somatic rearrangement is necessary. This is similar to antibody specificities, which reside predominantly in the CDR3 of the heavy chain (*14*, *15*). Also, in the case of αβ TCRs, peptide-MHC specificity is determined largely by CDR3α and CDR3β, but the nature of the antigen-recognition determinants of T22-specific γδ TCRs and αβ TCRs are quite different. The T22-specific CDR3δ motif is encoded mainly by Dδ2 with contributions from Vδ, Dδ1, and P nucleotides, whereas in αβ TCRs the most critical residues for peptide-MHC recognition are encoded either completely or partially by N nucleotides in both CDR3α and CDR3β (*15*).

To determine whether a largely intact Dδ2 is a unique feature of T22-specific TCRs or of γδ TCRs in general, we analyzed the Dδ2 length distribution of in-frame thymocyte TCRδ sequences (N = 431). We found that ~23% of these sequences contain Dδ2 in its entirety, whereas an additional ~30% retain at least 13 out of 16 Dδ2 nucleotides (Table 1). A similar Dδ2 length distribution was also found in nonselected TCRs (N = 271) consisting of out-of-frame TCRδ chains and TCRδ rearrangements from CD3ε$^{-/-}$ thymocytes, which cannot express surface TCR (Table 1). This indicates that TCRδ rearrangements are strongly biased toward maintaining long Dδ2 regions. In the periphery, more than 50% of both the T22-specific and non–T22-specific splenic and IEL sequences contain Dδ2 in its entirety, and more than 70% of the sequences have less than three nucleotides deleted (Table 1), indicating that the resulting

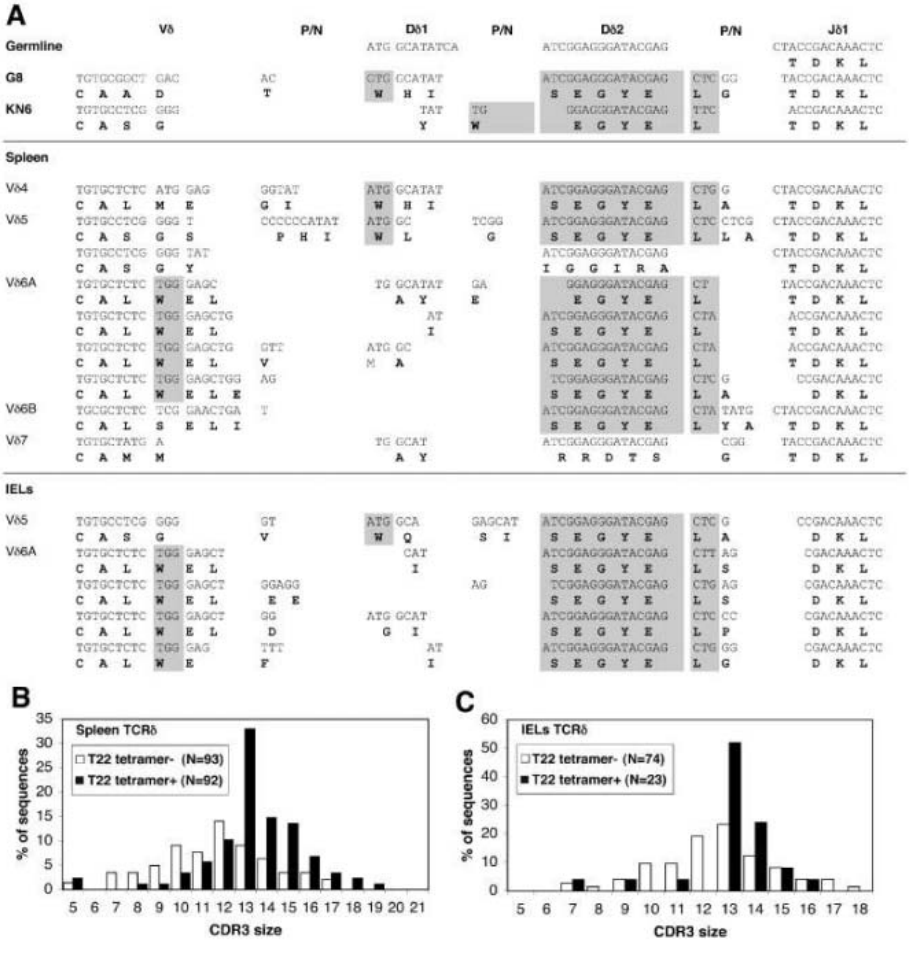

Fig. 2. (**A**) CDR3δ nucleotide and amino acid sequences from G8, KN6, and representative T22 tetramer-positive TCRs with the W-SEGYEL motif highlighted (*22*). CDR3δ size distributions for the (**B**) splenocyte and (**C**) IEL populations [calculated according to (*23*)] using productive rearrangements from the single cell sequence analyses (N is the total number of rearrangements analyzed).

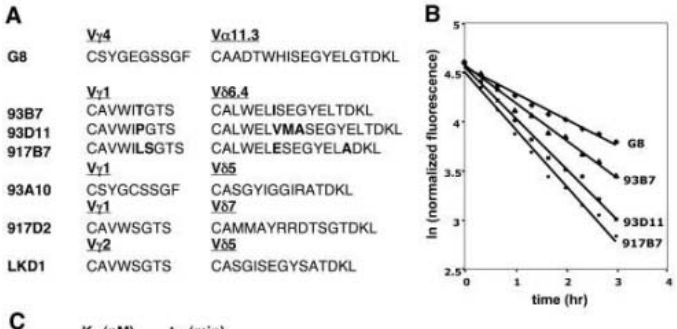

Fig. 3. (**A**) CDR3 sequences of G8, LKD1 (MHC class II I-A^d-specific), 93B7, 93D11, 917B7 (containing the W-SEGYEL motif), and 93A10 and 917D2 (not containing the motif) TCRs (*22*). The 93B7, 93D11, and 917B7 TCRs had two in-frame γ chain rearrangements (table S1). Only the sequence of the TCRγ chain that resulted in surface TCR expression is shown. Differences among the 93B7, 93D11, and 917B7 CDR3δ sequences are in bold type. (**B**) Tetramer decay plots are representative of three independent experiments. (**C**) The $t_{1/2}$ derived from the tetramer decay assay and the K_D determined by Scatchard analysis (*24*) are the average of three independent experiments ($P < 0.05$). The 93A10 and 917D2 TCR-expressing cells do not bind T22 tetramer (as indicated by nd).

TCRs are further selected for full use of the Dδ2 segment. In contrast, Dβ sequences from lymph node CD4+, Vβ17+ αβ T cells (*16*) show that only 3 to 7% are intact and fewer than 15 to 30% have been truncated by three nucleotides or less (Table 1).

Another feature distinguishing TCRδ CDR3 sequences from those of TCRβ and IgH chains is the J region. In both the TCRβ and the IgH chains, multiple J regions (12 Jβs and 6 J_Hs in mice) provide important framework residues and also contribute to antigen binding via their N-terminal residues (*15*). Exonuclease digestion and the addition of N nucleotides to the J region contribute to variability and thus to antigen binding (*15*). In contrast, adult murine γδ TCRs use only one Jδ, and the degree of exonuclease digestion is quite limited compared with αβ TCRs in that more than 98% of the sequences (T22-specific as well as non-specific) retain the first or second N-terminal amino acid residue encoded by Jδ1 (Table 2). This very limited J region diversity is also found among thymocytes and nonselected γδ TCRs (Table 2), revealing yet another unique feature of TCRδ gene rearrangement. This relative lack of variation suggests that, unlike J_H and Jβ, Jδ1 does not play a major role in antigen recognition.

Although most γδ T cell ligands have yet to be identified, our observations indicate that rearrangements at the TCRδ locus are largely biased toward full-length Dδ2 sequences rather than extensive D-region nucleotide deletion, as is the case for the TCRβ locus. Thus, different reading frames of Dδ2 may contribute to the recognition of other ligands by γδ TCRs in a manner similar to that of T22-specific γδ TCRs. This would allow these germ line–encoded CDR3 sequences to coevolve with their ligands. In fact, most well-defined γδ T cells' ligands are self-molecules that could act as indicators of physiological disturbances, such as T10 and T22 in the mouse and MICA and B, CD1, and F1–adenosine triphosphate synthase in humans (*3*, *17*–*19*).

One would expect that a T cell repertoire generated from somatic recombination but whose specificity is conferred by germ line–encoded amino acids (such as for T22-specific γδ TCRs) would be created much more frequently than αβ T cells whose specificity is conferred primarily by N-nucleotide additions. In fact, we find that 0.85% of nonselected TCRδ sequences (N = 353) contain this CDR3δ motif (table S5) compared to one in 10^5 to 10^6 αβ T cells specific for a given peptide-MHC before clonal expansion (*20*, *21*). Thus, rearrangement alone could in part account for the high frequency (0.1 to 2%) of T22-specific γδ T cells in normal mice (Fig. 1A) (*3*, *12*). If γδ TCR specificity for other ligands is determined in a similar manner, then the γδ T cell repertoire must be directed against a relatively small number of ligands but with high frequency. This could allow for a rapid and significant response without an initial need for clonal expansion.

The CDR3δ provides the TCRδ with the highest potential diversity of all antigen receptor polypeptides. The results described here show that this diversity endows T22-specific γδ TCRs with different ligand-binding affinities. Indeed, the T22-specific TCR repertoire in normal mice covers a range of affinities, as evidenced by the large range of T22 tetramer-staining intensities (Fig. 1) (*3*, *12*). A self-reactive TCR repertoire with such diverse ligand-binding properties would enable more flexible and efficient responses to changes in self-ligand expression and at the same time allow for selection against high-affinity T cells that might respond inappropriately to basal ligand expression amounts.

Table 1. Dδ2 length distribution in TCRδ rearrangements. Numbers represent the percentage of rearrangements with the indicated number of nucleotides removed. The lengths of D regions were analyzed in nucleotides because they can be read in all three reading frames. Sequences analyzed are functional T22 tetramer-positive and -negative TCRδ chains (tables S1 to 4); functional TCRδ chains from γδ T cell hybridomas (*25*) and thymocytes (*26*) nonselected TCRδ chains from CD3$\varepsilon^{-/-}$ thymocytes (*25*), out-of-frame rearrangements from γδ T cell hybridomas, and single-cell analyses from thymocytes (*27*); and CD4+, Vβ17+ TCRβ chains from the lymph nodes of SJL mice (*15*) (*n* indicates the number of sequences analyzed).

Dδ/Dβ nucleotides deleted	Spleen		IEL		Functional TCRδ chains (n = 431) (%)	Nonselected TCRδ chains (n = 271) (%)	Vβ17+ CD4+ αβTCR Dβ1 (n = 37) (%)	Vβ17+ CD4+ αβTCR Dβ2 (n = 57) (%)
	Tetramer+ (n = 92) (%)	Tetramer– (n = 93) (%)	Tetramer+ (n = 23) (%)	Tetramer– (n = 77) (%)				
0	55.4	36.5	52.2	44.6	23	21.4	2.7	7
1−3	29.3	30.1	30.4	25.7	30.2	28	10.8	21.1
4−6	8.7	19.4	8.7	20.3	22.5	22.5	51.3	28.1
7−10	6.5	12.9	4.3	9.4	23	22.5	0	21
undetermined	0	1.1	4.3	0	1.4	5.5	35	22.8

Table 2. Jδ1 length distribution in TCRδ rearrangements. Numbers represent the percentage of rearrangements with the indicated number of amino acids (J region) removed. Sequences analyzed are functional T22 tetramer-positive and -negative TCRδ chains (tables S1 to 4); functional TCRδ chains from γδ T cell hybridomas (*25*) and thymocytes (*26*) nonselected TCRδ chains from CD3$\varepsilon^{-/-}$ thymocytes (*25*), out-of-frame rearrangements from γδ T cell hybridomas, and single-cell analyses from thymocytes (*27*); and CD4+, Vβ17+ TCRβ chains from the lymph nodes of SJL mice (*15*) (*n* indicates the number of sequences analyzed).

Jδ/Jβ amino acids deleted	Spleen		IEL		Functional TCRδ chains (n = 431) (%)	Nonselected TCRδ chains (n = 271) (%)	Vβ17+ CD4+ αβTCR Jβ (n = 75) (%)
	Tetramer+ (n = 92) (%)	Tetramer– (n = 93) (%)	Tetramer+ (n = 23) (%)	Tetramer– (n = 77) (%)			
0	69.1	79.4	23.1	80.5	68.4	71.3	34.7
1	30.9	19.6	76.9	18.2	26	22.8	44
2	0	1	0	1.3	5.6	5.9	16
3 or more	0	0	0	0	0	0	7

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22. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Do 15-Month-Old Infants Understand False Beliefs?

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For more than two decades, researchers have argued that young children do not understand mental states such as beliefs. Part of the evidence for this claim comes from preschoolers' failure at verbal tasks that require the understanding that others may hold false beliefs. Here, we used a novel nonverbal task to examine 15-month-old infants' ability to predict an actor's behavior on the basis of her true or false belief about a toy's hiding place. Results were positive, supporting the view that, from a young age, children appeal to mental states—goals, perceptions, and beliefs—to explain the behavior of others.

Consider the following situation: A child who has surreptitiously eaten the last cookies in a box sees her brother reach into the box. To make sense of his behavior, she must understand that he falsely believes the box still contains cookies. As adults, we readily understand that others may hold and act on false beliefs; this ability is widely held to be a cornerstone of social competence, and its neuronal correlates have recently begun to be examined (*1*). What are the origins of this ability? Within the field of psychology, there has been a longstanding controversy regarding this issue (*2–4*).

Some researchers have suggested that at about 4 years of age a fundamental change occurs in children's understanding of others' behavior, or "theory of mind": They begin to realize that mental states such as beliefs are not direct reflections of reality, which must always be accurate, but representations, which may or may not be accurate (*5–8*). Part of the evidence for this change from a nonrepresentational to a representational theory of mind has come from young children's well-documented failure at false-belief tasks (i.e., tasks that require the understanding that others may hold and act on false beliefs) (*9–13*). In a standard task (*10*), children listen to a story as it is enacted with dolls and toys: The first character hides a toy in one location and leaves the room; while she is gone, a second character hides the toy in a different location. When asked where the first character will look for her toy, 4 year olds typically say she will look in the first location and provide appropriate justifications for their answers. In contrast, most 3 year olds say she will look in the second (actual) location, thus failing to demonstrate an understanding that the first character will hold a false belief about the toy's location.

Other researchers have suggested that a representational theory of mind is present much earlier and that young children's difficulties with the standard false-belief task stem primarily from excessive linguistic, computational, and other task demands (*14–18*). Support for these claims comes in part from evidence that 3 year olds and even some 2 year olds succeed at a modified false-belief task (*19*, *20*). In this version of the task, after listening to the story and watching it enacted, children are simply probed by the experimenter to look where the first character will search for her toy upon her return ("I wonder where she will look"). Most children look to the correct location, suggesting that they possess some implicit understanding that others may hold and act on false beliefs. We examined whether 15-month-old infants tested with a simpler, entirely nonverbal task would also show some implicit understanding of false belief.

We used the violation-of-expectation method, which has been used extensively to investigate infants' understanding of others' goals (*21–23*). For example, in one experiment (*22*), infants were familiarized with an actor reaching for and grasping one of two toys (defined as the target toy). Next, the locations of the two toys were reversed, and the actor reached for the target or the nontarget toy. The infants looked reliably longer at nontarget reaches. This and control results suggested that the infants encoded the target toy as the actor's goal object, expected her to reach for it in its new location, and responded with increased attention when she did not. Similar results were found when the target toy was hidden rather than visible and was retrieved by means-end action sequences rather than by a simple reach (*23*). Our research built on these results. In our experiment, 15-month-old infants first watched an actor hide a toy in one of two locations. Next, a change occurred that resulted in the actor holding either a true or a false belief about the toy's location. The experiment asked whether the infants would expect the actor to search for her toy based on her belief about its location, whether that belief was true or false.

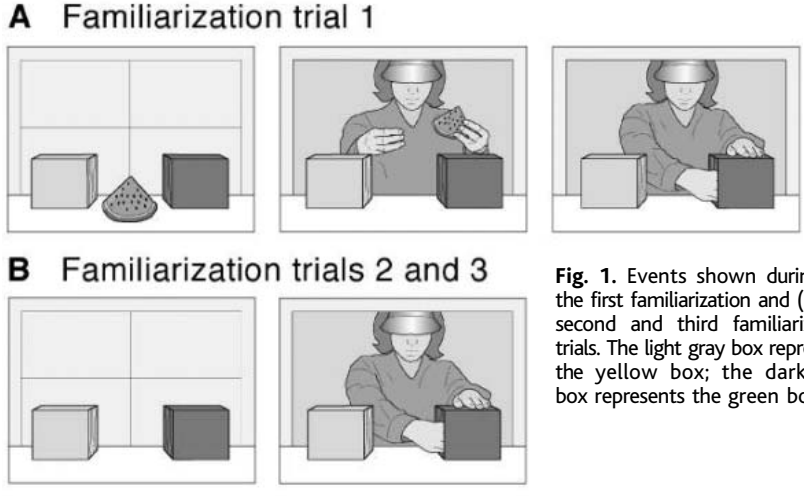

Fig. 1. Events shown during (**A**) the first familiarization and (**B**) the second and third familiarization trials. The light gray box represents the yellow box; the dark gray box represents the green box.

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The infants first received three familiarization trials (Fig. 1). At the start of the first trial, a toy watermelon slice rested on the apparatus floor between two boxes, one yellow and one green; the boxes' openings faced each other and were covered with fringe. An actor (wearing a beige visor and a denim shirt) opened doors in the back wall of the apparatus, grasped the toy, played with it for a few seconds, and then hid it inside the green box. After this pretrial, the actor paused, with her hand inside the box, until the trial ended (a curtain was lowered in front of the apparatus between trials). During the second and third familiarization trials, the actor opened the doors, reached inside the green box (as though to grasp the toy she had previously hidden there), and then paused until the trial ended. In all trials, looking times during the pretrial and paused portions of the trial were computed separately.

Next, the infants received a single belief-induction trial (Fig. 2). During this trial, the infants witnessed a change that resulted in the actor holding a true or a false belief about the toy's location. There were four versions of this trial, designed to yield two true-belief (TB) and two false-belief (FB) conditions: The actor could believe, truly or falsely, that the toy was hidden in the green or in the yellow box. In the TB-green condition, the actor was induced to have a true belief that the toy was in the green box. The upper halves of the doors in the back wall of the apparatus were open. The actor leaned into this opening and watched as the yellow box moved half the distance toward the green box and then returned to its original position; the infant and the actor thus observed no change in the toy's location and could assume that it remained in the green box. In the TB-yellow condition, the actor watched through the opening in the back wall as the toy moved from the green into the yellow box; thus, both the infant and the actor saw the toy change location. The FB-green condition was identical to the TB-yellow condition except that the opening in the back wall remained shut throughout the trial; because only the infant saw the toy move into the yellow box, the actor should have a false belief about the toy's location. The FB-yellow condition began as in the TB-yellow condition: The actor watched through the opening in the back wall as the toy moved from the green to the yellow box. Next, the actor shut the opening, and then the toy returned to the green box; thus, only the infant observed the toy's second displacement, leaving the actor with a false belief that the toy was still in the yellow box. In each condition, following the pretrial, the infants watched the final paused scene until the trial ended. All but five infants (distributed among three conditions) looked continuously during the pretrial, which lasted either 8 s (TB-green, TB-yellow, and FB-green) or 24 s (FB-yellow); the maximum time spent looking away for any individual was 0.6 s. The infants were thus very attentive throughout the pretrial.

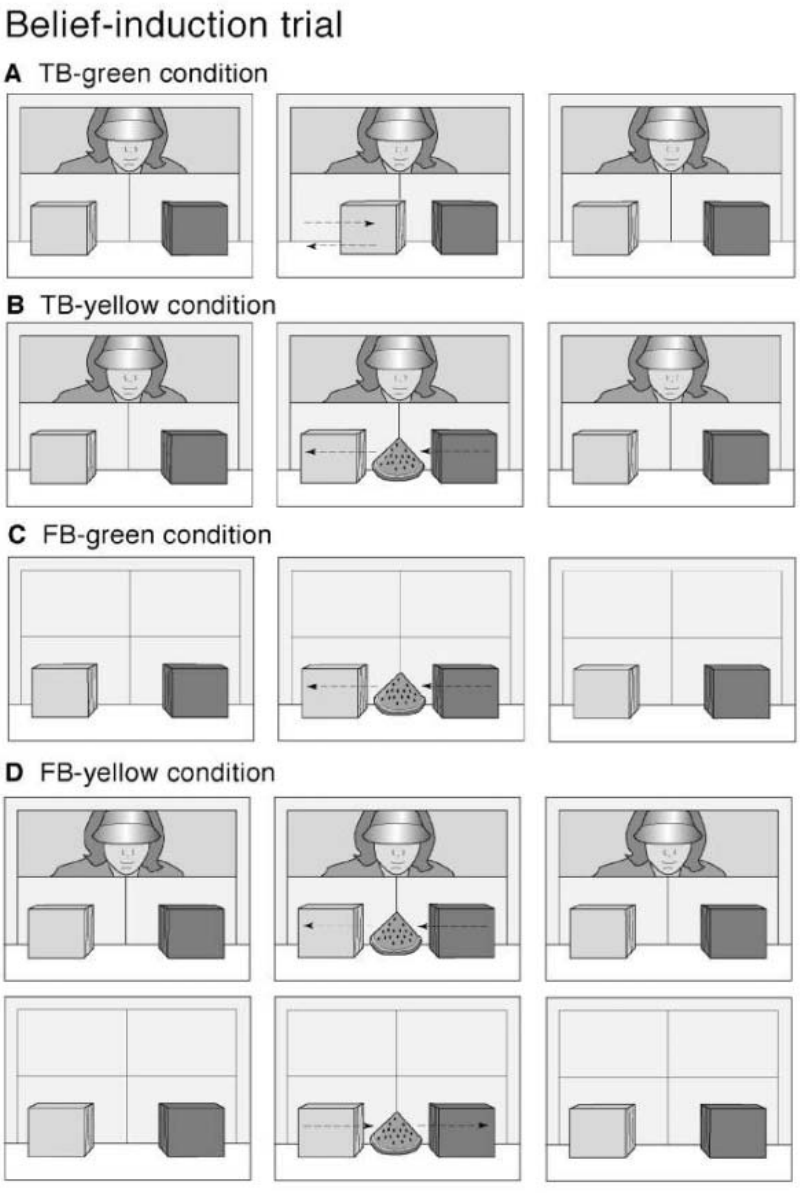

Fig. 2. Events shown during the belief-induction trial in the (**A**) TB-green condition, (**B**) TB-yellow condition, (**C**) FB-green condition, and (**D**) FB-yellow condition.

After the belief-induction trial, the infants received a single test trial (Fig. 3). For half of the infants in each belief condition, the actor opened the doors, reached into the green box, and paused until the trial ended (green-box condition); for the other infants, the event was the same except that the actor reached into the yellow box (yellow-box condition).

Our predictions for the test trial were as follows: If the infants expected the actor to search for her toy on the basis of her belief about its location, rather than on the basis of (their knowledge of) its actual location, then they should look reliably longer when that expectation was violated. Thus, when the actor had a true belief that the toy was hidden in the green box, the infants should expect her to reach into that box and they should look reliably longer when she reached into the yellow box instead; conversely, when the actor had a true belief that the toy was hidden in the yellow box, the infants should look reliably longer when she searched the green as opposed to the yellow box. Exactly the same predictions held when the actor had a false belief about the toy's location: The infants should look reliably longer at the yellow-box event when the actor falsely believed that the toy was hidden in the green box and at the green-box event when she falsely believed that the toy was hidden in the yellow box. Within both the true- and the false-belief conditions, an interaction was thus predicted between the actor's belief about the toy's location

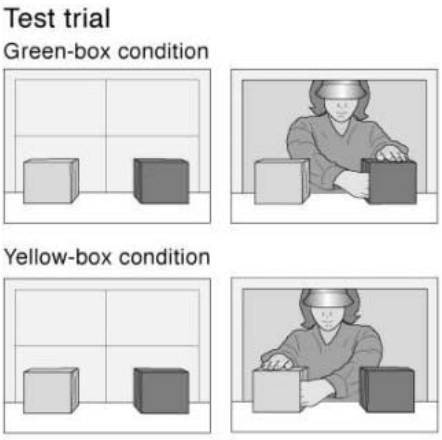

Fig. 3. Events shown during the test trial.

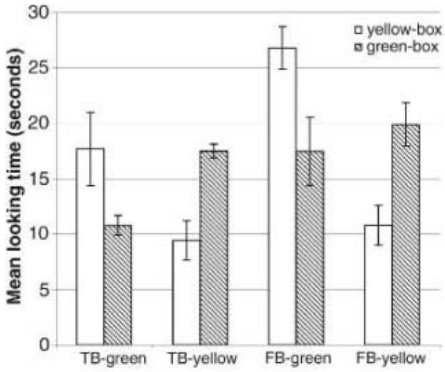

Fig. 4. Mean (±SE) looking times during the test trial (after the actor reached into the green or yellow box) in the four belief conditions.

and her action in the test: In each case, the infants should look reliably longer when the location the actor searched was inconsistent with her belief about the toy's location.

Participants were 56 healthy-term infants, 27 female and 29 male, with a mean age of 15 months, 7 days (range: 14 months, 27 days to 15 months, 18 days). Seven infants were randomly assigned to each of the eight groups formed by crossing the three experimental factors: the actor's belief about the toy's location (green or yellow box), the status of the actor's belief (true or false), and the location the actor searched during test (green or yellow box). Another 14 infants were tested but eliminated due to inattentiveness (4), looking more than 3 SD beyond the condition mean (4), fussiness (2), parental interference (2), or observer error (2) (*24*).

The infants' looking times during the test trial (Fig. 4) were compared by means of an analysis of variance with actor's belief about the toy's location (green or yellow box), belief status (true or false), and actor's action (green or yellow box) as between-subject factors. The predicted interaction between actor's belief and actor's action was reliable [$F(1, 48) = 31.24$, $P < 0.0001$], indicating that the infants expected the actor to reach where she believed the toy to be and looked longer when she did not. This interaction was also reliable within the true-belief [$F(1, 24) = 14.49$, $P < 0.0008$] and the false-belief [$F(1, 24) = 16.69$, $P < 0.0004$] conditions. Finally, planned comparisons indicated that, in each of the four belief conditions, infants expected the actor to search for her toy where she believed it to be hidden and looked reliably longer when she did not (for all conditions, $F > 5.34$, $P < 0.05$) (see supporting online material text for analyses).

Whether the actor believed the toy to be hidden in the green or the yellow box and whether this belief was in fact true or false, the infants expected the actor to search on the basis of her belief about the toy's location. These results suggest that 15-month-old infants already possess (at least in a rudimentary and implicit form) a representational theory of mind: They realize that others act on the basis of their beliefs and that these beliefs are representations that may or may not mirror reality.

Could our results be explained in terms of low-level strategies the infants might have used to predict the actor's behavior? Together, the four conditions demonstrate that the infants did not simply expect the actor to search where the toy was actually hidden (FB-green and FB-yellow), where she had previously searched (TB-yellow and FB-yellow), or where she had last attended (TB-green). In addition, the results make clear that the infants did not simply become confused when the actor held a false belief and expect her to repeat whatever action she had last performed (FB-yellow).

Could the infants have used a more sophisticated strategy that still fell short of attributing to the actor a belief about the toy's location? Perhaps the infants brought to the task a superficial expectation (acquired through repeated observations) that a person looking for an object will search for it where she last saw it disappear. This interpretation (which could also be offered for the modified false-belief task described earlier) assumes that the infants (i) distinguished between their own and the actor's perceptions; (ii) kept track of what the actor did and did not see; and (iii) understood that the actor's perceptions (rather than their own) should be used to predict her behavior. On this interpretation, our research would add to previous findings on the ability of young children to keep track of others' perceptions. For example, 2.7 year olds kept track of whether their parent was present or absent when a toy was hidden in a room; if the parent was absent, children were more likely to point to the toy's location when the parent returned (*25*). According to this alternative interpretation, our research would extend these results by showing that 15-month-old infants respond appropriately even when the actor is mistaken, as opposed to simply ignorant, about the toy's location and even when this information must be used to predict the actor's behavior rather than guide their own.

We prefer our interpretation to the alternative interpretation just discussed for two reasons. The first is theoretical. Similar to other researchers (*14*–*18*), we assume that children are born with an abstract computational system that guides their interpretation of others' behavior. In this view, even young children appeal to others' mental states—goals, perceptions, and beliefs—to make sense of their actions; development involves primarily learning which states underlie which actions and not coming to understand that such states exist at all. The second reason is empirical. Recent results of ours have indicated that infants can predict where an actor will search for a hidden toy even when she does not see it disappear but must infer its location based on various (useful or misleading) cues (*26*, *27*). To explain these and the present results, it is more parsimonious to assume that infants attribute to others beliefs that can be shaped and updated by multiple sources of information than to assume that infants form an extensive series of superficial expectations linking different perceptions to different actions. In short, we propose that the present results suggest that 15-month-old infants expect an actor to search for a toy where she believes, rightly or wrongly, that it is hidden. Such an interpretation calls into question the notion that preschoolers undergo a fundamental change from a nonrepresentational to a representational theory of mind.

Beyond these immediate conclusions, the present findings have potential implications for two fields of research. The first is atypical development. Autistic children generally fail standard false-belief tasks and as a result are often described as possessing a deficient theory of mind (*10*, *28*). If nonverbal false-belief tasks could be adapted for use with this population, it would open new avenues of research into the nature and early detection of autism. The second field of research is that of animal cognition. Since the pioneering work of Premack and Woodruff (*29*), the issue of whether animals possess a theory of mind has attracted much attention (*30*). The development of various nonverbal false-belief tasks may lead to new insights in the field of animal cognition (*31*).

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Assortative Mating in Sympatric Host Races of the European Corn Borer

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Although a growing body of work supports the plausibility of sympatric speciation in animals, the practical difficulties of directly quantifying reproductive isolation between diverging taxa remain an obstacle to analyzing this process. We used a combination of genetic and biogeochemical markers to produce a direct field estimate of assortative mating in phytophagous insect populations. We show that individuals of the same insect species, the European corn borer *Ostrinia nubilalis*, that develop on different host plants can display almost absolute reproductive isolation—the proportion of assortative mating was >95%—even in the absence of temporal or spatial isolation.

The evolution of host races in phytophagous insects provides opportunities for studying processes that may ultimately lead to sympatric speciation (*1–3*). In the absence of geographic isolation, postzygotic barriers may contribute to genetic differentiation, but the key mechanism ensuring reproductive isolation over time is assortative mating (*4*). This mechanism may be selected per se, via reinforcement (*5*), or as a by-product of host specialization (pleiotropy) (*6*).

Despite its pivotal function in speciation, the overall level of assortative mating between sympatric populations has never been quantified directly in natural populations of phytophagous insects. First, it is often difficult to detect individuals in the field at the very moment they mate. Second, morphological or genetic markers often provide insufficient resolution for the assignment of individuals to host races. Hence, even the most comprehensive studies on insect host races (*7–12*) provided indirect estimates of assortative mating. These estimates were obtained from experiments carried out in the laboratory or with laboratory-reared individuals (*8–10*), and/or derived from the measurement of factors thought to favor assortative mating, such as temporal isolation (*7*) and host fidelity (*8*, *11*, *12*). However, assortative mating probably results from flexible behavioral decisions influenced by interactions between physiological and environmental factors (e.g., related to host plants).

A combination of genetic (*13*, *14*) and biogeochemical (*15*, *16*) markers was used to produce a global, direct quantitative field estimate of assortative mating between sympatric phytophagous insect host races. Populations of the European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), feed on more than 200 weeds and cultivated plants (*16*) and must have colonized maize (*Zea mays* L.) after its introduction into Europe, about 500 years ago. In France, ECB populations can be separated into at least two genetically different taxa that exhibit all core characteristics (*13*, *16–20*) of host races (*3*): the so-called "hop-mugwort-E race," which feeds on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.) and communicates with the "E" blend of sex pheromone isomers; and the "maize-Z race," which feeds on maize and communicates with the "Z" pheromone blend (*13*, *17*, *19–21*). Although both races display host fidelity for oviposition (*18*), mating does not occur primarily on the host plant itself but on various other species, sometimes several hundred meters from the nearest host plant (*22*). As for most model taxa in host-race studies (*1–3*), it is uncertain whether ECB host races originally diverged in sympatry. However, they presently co-occur over a large geographical range (*17*, *20*). Thus, they can provide insight into how genetic differentiation is maintained in sympatry, a necessary condition for sympatric speciation. Some hybrids between ECB host races were obtained in experimental settings (*18*) and some pheromonal hybrids were found in natura (*20*), but both were rare, pointing at assortative mating as a key mechanism maintaining genetic differentiation. The level of assortative mating between host races in the field had, however, never been quantified.

Over 2 weeks of the July 2002 breeding season, we caught a total of 417 moths at five sites located within 4 km of each other. Their wings were subjected to stable carbon isotope analysis (*23*). Because the $\delta^{13}C$ value of animal tissues closely mirrors that of the animal's food (*24*), ECB adults that emerged from larvae fed on the two types of host plant can be distinguished according to the $\delta^{13}C$ value of their tissues (*16*, *19*). The $\delta^{13}C$ values obtained here showed a bimodal distribution, with no overlap (Fig. 1A). Values of −31 to −22‰ are typical for individuals feeding on plants with C_3 photosynthesis such as mugwort and hop, whereas values of −19 to −9‰ are typical of individuals feeding on C_4 plants such as maize (*16*, *24*). This method therefore resulted in the unambiguous assignment of each moth to one of the two host-plant types.

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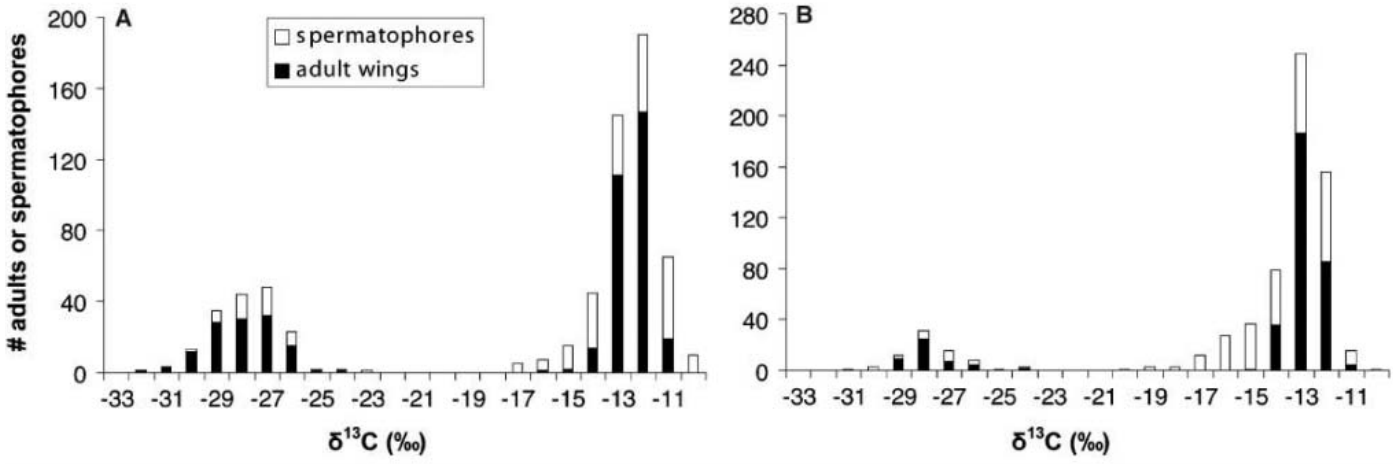

Fig. 1. Frequency of $\delta^{13}C$ values (‰) in (**A**) 2002 and (**B**) 2003, for wings and spermatophores.

Moths with a C_3-type $\delta^{13}C$ (n = 152, 36.5%) will be referred to as "C_3 moths," and those with a C_4-type $\delta^{13}C$ (n = 265, 63.5%) as "C_4 moths." We carried out genetic analyses (*23*) on these moths and on larvae collected from maize, hop, and mugwort (Fig. 2). We found that populations of C_3 and C_4 moths displayed genetic profiles typical of the hop-mugwort-E and maize-Z races, respectively. This finding is consistent with the facts that (i) both races display strong host fidelity for oviposition (*18*); (ii) a previous field survey (*20*) showed that all females (except three hybrids, n = 345) collected at larval stage on maize and hop/mugwort produced the Z and E pheromone, respectively; and (iii) most adult males caught with E- and Z-baited pheromone traps proved to be C_3 and C_4, respectively, and displayed the corresponding genetic allozyme profiles (*19*).

The five study sites differed considerably in the relative proportions of C_3 and C_4 moths (Table 1), indicating differences in the fine-scale spatial distribution of both host races. However, the two races were truly sympatric in sites 3, 4, and 5 (Table 1), providing ample opportunity for hybrid matings. The $\delta^{13}C$ values of the 237 spermatophores (*25*) showed a bimodal distribution very similar to that of the wings (Fig. 1A), allowing the unequivocal assignment of each female's partner to the C_3 or C_4 group (*16*). All but one of the C_3 females (n = 44) had mated with C_3 males, and all but one of the C_4 females (n = 142) had mated with C_4 males (Table 1). Overall, both ECB host races displayed very similar and very high (~95%) proportions (*23*) of assortative mating (Table 2).

A previous study in the same geographical area showed that, on average, moths of the hop-mugwort-E race emerged earlier than those of the maize-Z race (*17*), increasing the probability of assortative mating. Therefore, during the 2003 season, we checked for time lags between the flight peaks of C_3 and C_4 moths at four sites located in the same area. At

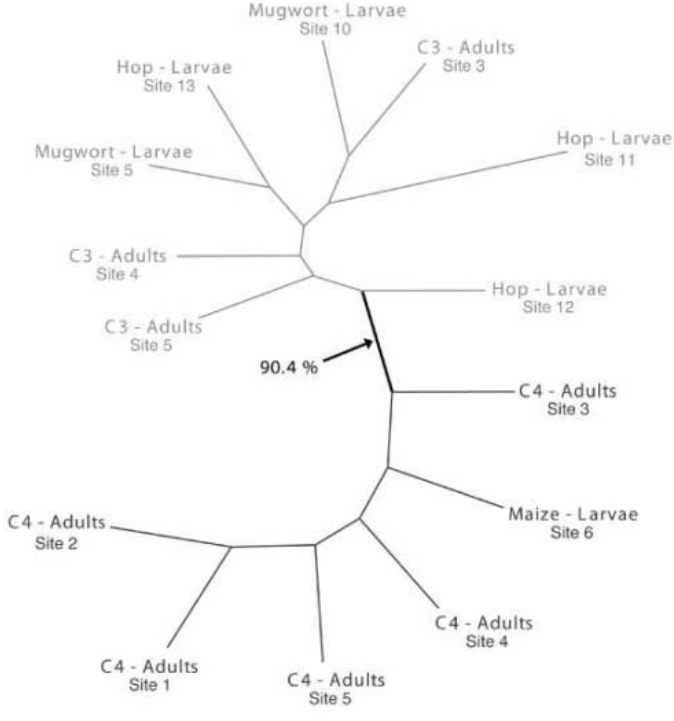

Fig. 2. Unrooted dendrogram inferred from genetic distance among populations. The position of the populations of C_4 moths and larvae collected on maize (a C_4 plant) relative to that of populations of C_3 moths and larvae collected on mugwort and hop (C_3 plants) is supported by a bootstrap value of 90.4% (10,000 resamplings). Populations of C_4 moths were not significantly differentiated from each other or from the population of diapausing larvae collected on maize [$0 < Fst < 0.004$; $P > 0.400$ for all comparisons (*23*)]. Conversely, they were significantly differentiated from C_3 moth populations and from populations of diapausing larvae collected on mugwort and hop ($0.041 < Fst < 0.132$; $P < 10^{-5}$ for all comparisons).

Table 1. Study sites, proportion of individuals that fed on a C_3 plant as larvae, and host-plant type of each female's partner, during the two study years.

Year	Site	Habitat	Males		Females		C_3 females' matings		C_4 females' matings	
			n	%C_3	n	%C_3	n mated females	% $C_3 \times C_3$	n mated females	% $C_4 \times C_4$
2002	1	Barley	50	0	53	4	2	50	48	100
	2	Maize	19	0	18	6	1	100	16	94
	3	Hop + maize	55	20	65	13	7	100	46	100
	4	Mugwort	57	46	42	39	15	100	23	100
	5	Mugwort	40	85	27	95	19	100	1	100
2003	6	Maize	69	12	48	10	5	100	43	100
	7	Maize	76	32	53	23	11	100	40	100
	8	Barley	12	0	23	0	0	–	22	100
	9	Barley	18	6	64	0	0	–	62	98

Table 2. Estimated proportion (mode and quantiles) of assortative mating for moths of both carbon types for all sites in 2002, 2003, and for both years together.

Year	Proportion of assortative mating					
	C_3 moths			C_4 moths		
	mode	q0.025	q0.975	mode	q0.025	q0.975
2002	0.949	0.741	0.988	0.961	0.771	0.990
2003	0.983	0.732	0.998	0.965	0.783	0.991
Both years	0.970	0.832	0.993	0.964	0.857	0.989

each site, we sampled insects once per week over 5 weeks, covering most of the flight period. We caught a total of 363 individuals. Again, the $\delta^{13}C$ values of the wings and of the 298 spermatophores carried by the females (Fig. 1B) made it possible to determine host-plant type unambiguously.

As in the 2002 sampling, the four sites differed in the relative proportions of C_3 and C_4 moths (Table 1). Unexpectedly, we found no evidence of temporal isolation between races in terms of flight periods. Indeed, the null hypothesis of homogeneity of C_3:C_4 proportions over time was not rejected at sites 7, 8, and 9, and there was no clear trend over time at site 6 despite rejection of the null hypothesis at this site (table S1). Hence, the two host races occurred together for a period of at least 5 weeks, covering almost the entire flight period. At all four sites, only one (C_4) female (n = 16 C_3 and n = 167 C_4 females) mated with a male that had not developed on the same type of host plant as herself (Table 1). Our 2003 results therefore confirm the very strong assortative mating observed in 2002 (Table 2). In both years, the maximum-likelihood estimates of the proportion of assortative mating in the C_3 and in the C_4 races in our study area were >95%, with 95% credibility intervals of 83.2 to 99.3% and 85.7 to 98.9%, respectively (Table 2). Interestingly, all three apparently hybrid matings observed in this study occurred in sites with C_3:C_4 moth ratios far from 1:1, as expected under assortative mating, rather than in sites with a ratio close to 1:1, as expected under random mating (Table 1).

Our estimate of hybridization frequency (<5%) is of the same order of magnitude as that of gene flow (<1%) based on the pattern of genetic differentiation between the hop-mugwort-E and maize-Z populations (*13*). Assortative mating, rather than postzygotic isolation, therefore appears to be the main cause of genetic differentiation in the ECB. Our results provide an example of the maintenance of very strong assortative mating even in the absence of spatial or temporal isolation. Measuring the net result of all factors influencing assortative mating in natura was possible here because *O. nubilalis* host races happen to feed on two isotopically different host-plant groups. Beyond this practical feature, our results reveal two additional reasons for which the ECB is a particularly interesting and suitable model to study assortative mating and other processes involved in sympatric speciation: (i) It displays a relatively high level of assortative mating compared with other host races; and (ii) this assortative mating appears to be strongly driven by factors not directly related to host-plant adaptation, e.g., sex pheromones (*20*). Both are somewhat rare cases among host races (*1–3*). Therefore, studies on assortative mating in ECB host races could pave the way to dissecting the relative contribution of various factors involved in reproductive isolation, as well as pre- and postzygotic barriers to gene flow.

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Table S1
References and Notes

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The Floral Regulator LEAFY Evolves by Substitutions in the DNA Binding Domain

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The plant-specific transcription factor LEAFY controls general aspects of the life cycle in a basal plant, the moss *Physcomitrella patens*. In contrast, LEAFY has more specialized functions in angiosperms, where it specifically induces floral fate during the reproductive phase. This raises the question of a concomitant change in the biochemical function of LEAFY during the evolution of land plants. We report that the DNA binding domain of LEAFY, although largely conserved, has diverged in activity. On the contrary, other, more rapidly evolving portions of the protein have few effects on LEAFY activity.

LEAFY (LFY) is found in all land plants, which evolved during the past 400 million years. The proteins are remarkably well conserved with two blocks of similarity, in the N- and C-terminal regions (the N and C domain) (Fig. 1A). All missense mutations

identified in *Arabidopsis* mutant screens map to these two domains, which make up a little more than half of the protein (table S1 and fig. S1). Between nonflowering and flowering plants, there is very little sequence conservation outside the N and C domains. In *Arabidopsis*, the nuclear LFY protein binds sequences in the enhancers of several floral homeotic genes, including *APETALA1* (*AP1*) (*1*–*4*). The missense changes found in mutant alleles very much reduce in vitro DNA binding to the *AP1* promoter (fig. S1). Deletion analyses identify a minimal DNA binding domain from amino acids 320 to 507 (numbering refers to consensus sequence), which includes the highly conserved C domain (Fig. 1A and fig. S1). Although the N domain is not essential for DNA binding, DNA binding is compromised in a deletion derivative that retains part of the N domain, as well as in a protein with a point mutation in the N domain. These observations suggest that the N domain regulates the activity of the DNA binding domain proper (fig. S1).

There is generally only a single copy of LFY in angiosperms (Fig. 1B). In species with lineage-specific duplications, these do not seem to have diverged in function (*5*, *6*). Nonflowering plants appear to have additional copies, but these are also all closely related and there is no evidence for major subfunctionalization (*7*, *8*). The strong sequence conservation of the DNA binding domain suggested that the molecular function of LFY is conserved as well. To test this assumption, we linked *LFY* cDNAs from 14 species to the *Arabidopsis LFY* promoter and introduced them into a strong *lfy* mutant. The 14 species represent the three main taxa with known LFY homologs: ferns and mosses, gymnosperms, and angiosperms (Fig. 1B).

Angiosperm genes fully complement *lfy* mutant, whereas gymnosperm genes provide only partial rescue (Fig. 2A), with *PRFLL* from pine (*9*) having more activity in this assay than WelNDLY from *Welwitschia* (*7*) (table S3). The *LFY* and *NDLY* clades represent a gymnosperm-specific duplication event, with the *NDLY* lineage having been lost in angiosperms (*10*). Among homologs from the most basal group, the fern genes *CrLFY2* and *AlLFY4* (*8*) have some rescue ability, although less than the gymnosperm genes, whereas the moss genes *PpLFY1* and *PpLFY2* (*8*) are inactive. This gradient of complementation reflects the phylogenetic distance from angiosperms (Fig. 1B) and suggests that a continuum of discrete and nonneutral changes, rather than a sudden modification, is responsible for changes in function.

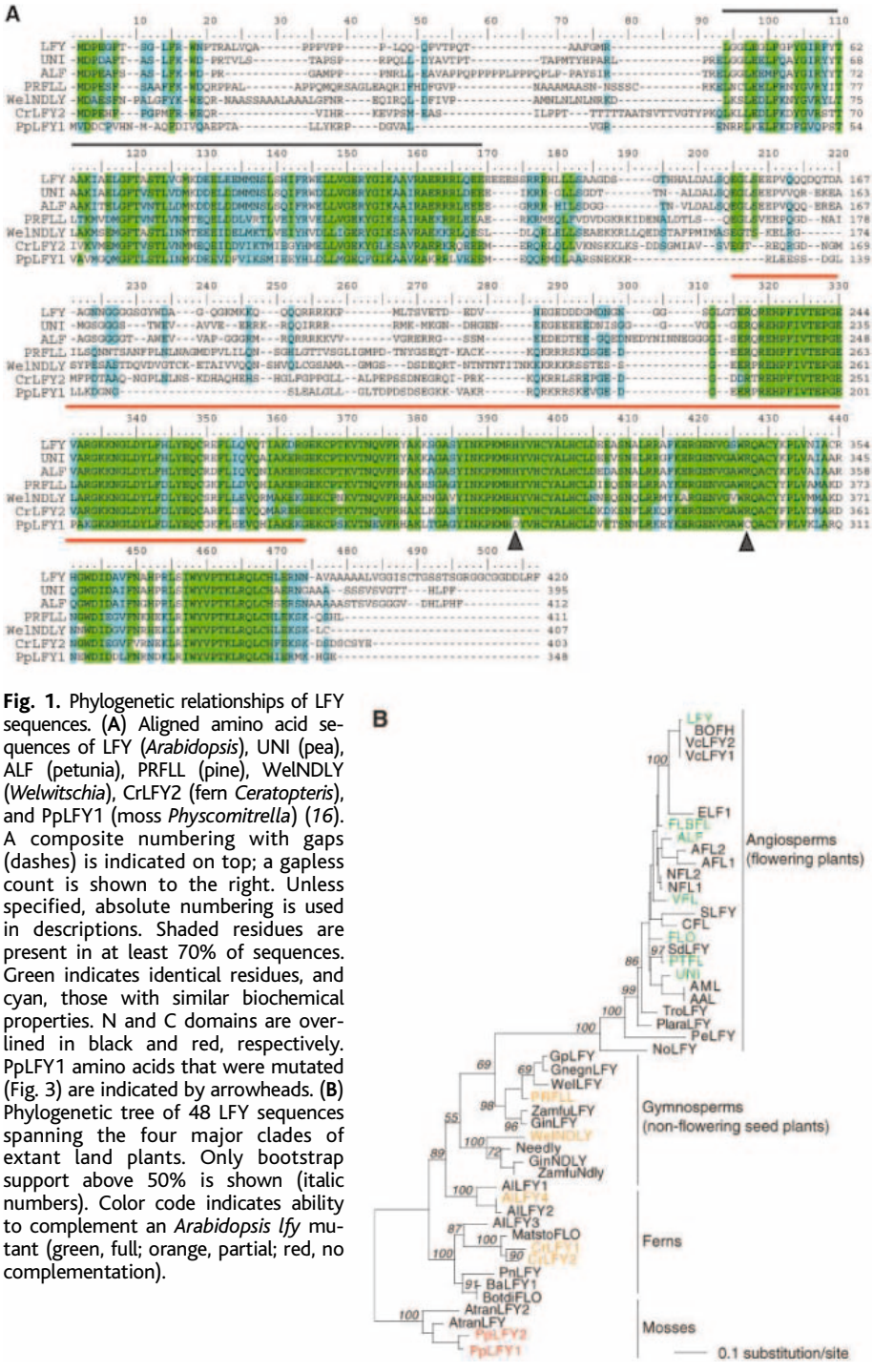

Fig. 1. Phylogenetic relationships of LFY sequences. (**A**) Aligned amino acid sequences of LFY (*Arabidopsis*), UNI (pea), ALF (petunia), PRFLL (pine), WelNDLY (*Welwitschia*), CrLFY2 (fern *Ceratopteris*), and PpLFY1 (moss *Physcomitrella*) (*16*). A composite numbering with gaps (dashes) is indicated on top; a gapless count is shown to the right. Unless specified, absolute numbering is used in descriptions. Shaded residues are present in at least 70% of sequences. Green indicates identical residues, and cyan, those with similar biochemical properties. N and C domains are overlined in black and red, respectively. PpLFY1 amino acids that were mutated (Fig. 3) are indicated by arrowheads. (**B**) Phylogenetic tree of 48 LFY sequences spanning the four major clades of extant land plants. Only bootstrap support above 50% is shown (italic numbers). Color code indicates ability to complement an *Arabidopsis lfy* mutant (green, full; orange, partial; red, no complementation).

We used microarrays to investigate in more detail the different activities of the homologs. Floral development was synchronized by transfer of plants from short days to long days, which induces *LFY* promoter activity (*11*, *12*). Of the 16 genes responding most strongly to *Arabidopsis LFY*, 15 and 13 are significantly induced by the angiosperm homologs *UNI* and *ALF*, respectively. *WelNDLY*, a gymnosperm representative, induces two targets, whereas *CrLFY2*, a fern gene, induces only one target. None of the *LFY* targets respond to *PpLFY1* from moss (Fig. 2B and fig. S2).

For orthologs of animal HOX proteins, altered activity in cross-species experiments

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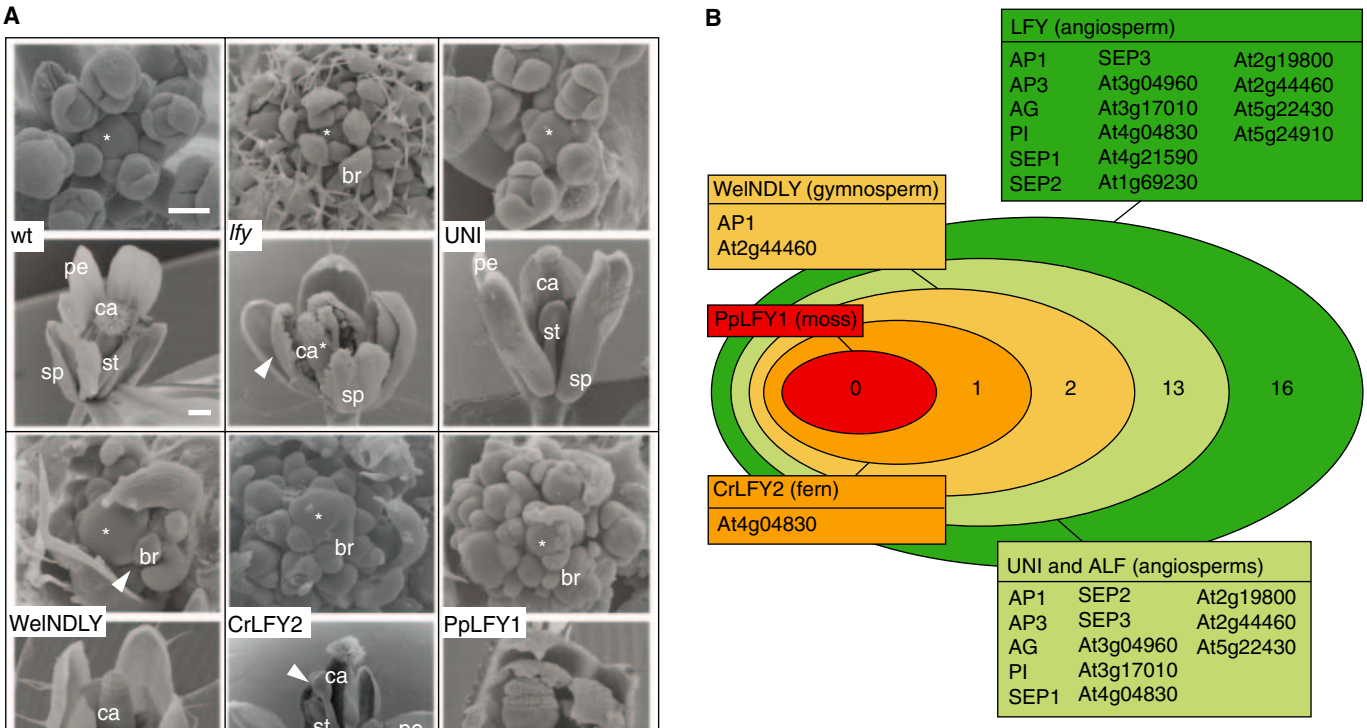

Fig. 2. Rescue of *Arabidopsis lfy* mutants by *LFY* homologs. (**A**) Scanning electron micrographs of inflorescence meristems (top) and mature flowers (bottom). In wild type (wt) and plants expressing pea *UNI*, the inflorescence meristem (asterisk) produces floral meristems, which differentiate into flowers composed of sepals (se), petals (pe), stamens (st), and carpels (ca). Plants expressing moss *PpLFY1*, like nontransgenic *lfy* mutants, have bracts (br) subtending secondary inflorescences, which replace early-arising flowers (arrowhead). Later-arising flowers consist of organs with mixed sepal and carpel identity (arrowhead) and unfused carpels (ca*). *Welwitschia WelNDLY* and fern *CrLFY2* partially rescue this phenotype. Later-arising flowers of *lfy WelNDLY* plants have an almost wild-type complement of organs, whereas *CrLFY2* flowers have spirally arranged organs that include petaloid stamens (arrowhead). Scale bar is 200 μm. (**B**) Synthetic Venn diagram of genes induced by *Arabidopsis* LFY. Ellipses represent the sets of genes induced by the different homologs (see Fig. 1B for color code).

has been traced back to changes outside the DNA binding domain proposed to affect the transcriptional activation or repression potential (*13*–*15*). To determine whether a similar scenario applies to LFY, we fused LFY homologs to the VP16 activation domain and tested whether they could, like an *Arabidopsis* LFY-VP16 fusion, induce expression of yeast reporters under the control of LFY binding sites from the homeotic genes *AP1* or *AGAMOUS* (*AG*) (*1*, *2*). The ability of the different VP16 fusions to interact with the *AP1* and *AG* sites and activate the yeast reporters parallels their rescue activity in plants. Of the proteins from nonflowering plants, PRFLL is as effective as *Arabidopsis* LFY, whereas WelNDLY and CrLFY2 are substantially less active. Moss PpLFY1, which is inactive in the transgenic plant assay, is also inactive in yeast, indicating that the failure to complement *Arabidopsis lfy* mutants is not simply caused by a change in transcriptional activation potential (Fig. 3A and fig. S3). Thus, in an a-minima model, declining ability to replace *Arabidopsis* LFY in plants is caused by a progressive failure to interact with canonical LFY binding sites.

Because the conserved N and C domains had been implicated in DNA binding, we suspected that the changes in activity are caused by divergence in these two domains, rather than by changes in the surrounding sequences. To test this hypothesis, we swapped the N and C domains between *Arabidopsis* LFY and CrLFY2 (Fig. 3B). Across the entire sequence, the LFY-CrLFY2 chimera, in which the N and C domains are derived from fern CrLFY2, is more similar to *Arabidopsis* LFY than the CrLFY2-LFY chimera (77% versus 67% sequence identity to *Arabidopsis* LFY). Nevertheless, it is the less-similar CrLFY2-LFY chimera that provides almost complete rescue when introduced into *Arabidopsis lfy* mutants, whereas the other chimera has very little activity, comparable to CrLFY2 itself (Fig. 3B). Thus, changes in the highly conserved N and C domains are responsible for most of the functional differences between the proteins from fern and *Arabidopsis*. We confirmed that these differences were caused by differential DNA binding activities with the yeast assay described above (fig. S4). Combination of the N and C halves of *Arabidopsis* LFY and CrLFY2 showed that the C domain, which corresponds to the minimal DNA binding domain, is primarily responsible for the divergence in function (fig. S5).

We found two amino acid substitutions, $His^{394} \rightarrow Asp^{394}$ (H394D) and $Arg^{427} \rightarrow Cys^{427}$ (R427C) (*16*), which discriminate between the DNA binding domains of proteins at opposite ends of the functional spectrum, *Arabidopsis* LFY and moss PpLFY1. We created versions of PpLFY1 in which these positions were individually changed to the angiosperm sequence and tested them again in the yeast assay. PpLFY1 (D394H) but not PpLFY1 (C427R) partially activated transcription (Fig. 3C and fig. S3). Similarly, in transgenic plants PpLFY1 (D394H) but not PpLFY1 (C427R) provided partial *LFY* activity (Fig. 3D). That a one–amino acid change is sufficient to have PpLFY1 bind a canonical LFY binding site indicates that position 394 is crucial for DNA binding.

The D394H substitution appears to be restricted to true mosses (*10*) and is not found in the liverworts *Riccia* and *Marchantia*. We consider it unlikely that the aspartate (D) at position 394 in moss PpLFY1 simply inactivates the protein, because moss

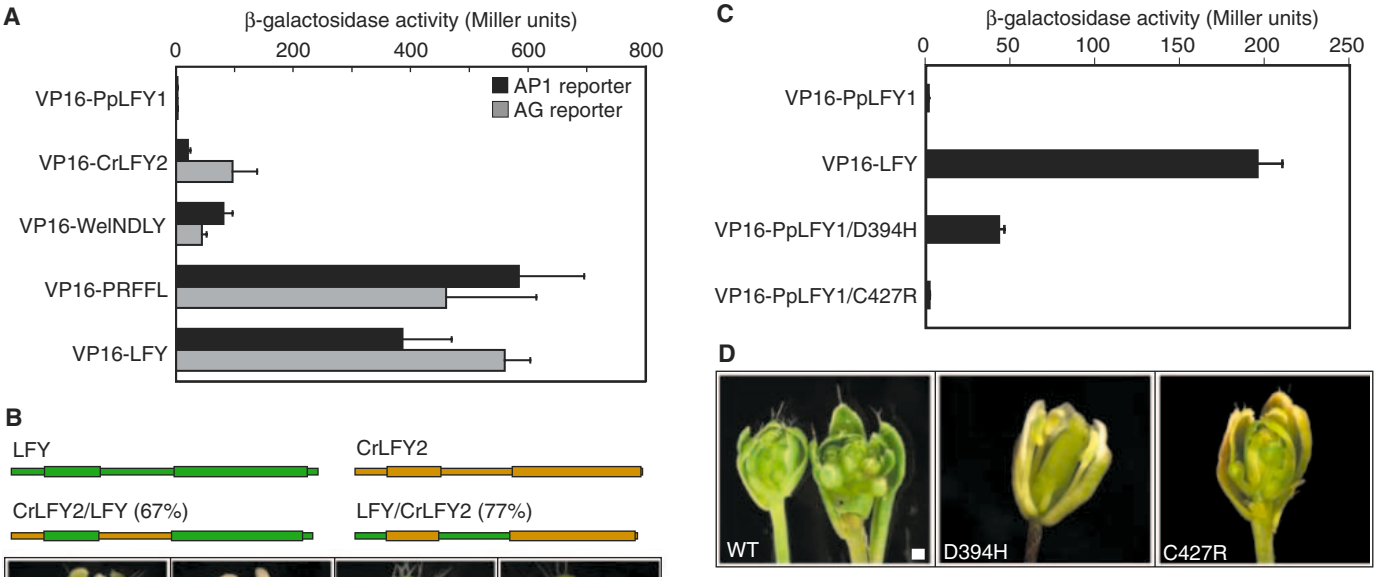

Fig. 3. Mapping of differences in activity of LFY homologs to DNA binding domain. (**A**) DNA binding activity of LFY proteins measured in a yeast reporter assay with the use of reporters containing trimerized binding sites from *AP1* or *AG* (*1*, *22*). Error bars indicate standard deviation. (**B**) In vivo activity of chimeric proteins. Chimeras of *Arabidopsis* LFY (green) and fern CrLFY2 (orange) are shown as diagrams on top, with conserved N and C domains shown as boxes; sequence identity with *Arabidopsis* LFY is indicated. Below, partially dissected flowers of *lfy* mutants expressing the different sequences are compared. Scale bar is 200 μm. (**C**) DNA binding affinity of LFY, PpLFY1, and PpLFY1 mutants assayed in yeast with the *AP1* binding site. (**D**) Images of *lfy* plants expressing wild-type *PpLFY1* (WT) or *PpLFY1* with D394H and C427R substitutions. Organs with petaloid and stamenoid characters are only seen with the D394H mutant. Scale bar is 200 μm.

plants lacking both *PpLFY* genes have dramatic developmental defects (*17*). We therefore conclude that PpLFY1 has most likely a different DNA binding specificity than its angiosperm counterpart (*10*). The gradient of activity among nonflowering plants furthermore suggests a systematic change in the DNA binding specificity of LFY homologs.

In animals, the relationship between molecular evolution of developmental regulators and morphological changes is best understood for HOX and PAX homeodomain proteins. Modification of expression patterns can lead to changes in morphology (*18*), but changes in the proteins themselves may be important as well. Functional differences in the activity of HOX orthologs from different species map to the repression and activation domains rather than the DNA binding domain (*13*, *14*). In contrast, our results emphasize the LFY DNA binding domain as a source of functional variation across species. Examples of molecular evolution of transcription factors in flies and worms that implicated the DNA binding domains as the source of variation have been interpreted as divergence within families of related multicopy genes. Gene duplication followed by subfunctionalization (*19*), as described for the PAX family (*20*), is unlikely to apply to LFY, because there has been no radiation of the *LFY* family during the evolution of land plants.

In a broader perspective of functional evolution of LFY among land plants, two scenarios can be considered. LFY might control similar networks of genes in nonflowering and flowering plants, with coevolution of target sequences and LFY DNA binding specificity. If this is the case, one still needs to postulate that these networks have been modified, because the primary target of LFY in angiosperms, *AP1*, is restricted to flowering plants (*21*). Alternatively, there may have been a complete change of LFY function between basal taxa and flowering plants, in which an initial, albeit gradual change in biochemical activity was the prerequisite for recruitment and/or intercalation of new targets, such as *AP1*.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5719/260/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S4
References

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Bivoltinism as an Antecedent to Eusociality in the Paper Wasp Genus *Polistes*

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To learn the evolutionary trajectories of caste differentiation in eusocial species is a major goal of sociobiology. We present an explanatory framework for caste evolution in the eusocial wasp genus *Polistes* (Vespidae), which is a model system for insect eusocial evolution. We hypothesize that *Polistes* worker and gyne castes stem from two developmental pathways that characterized the bivoltine life cycle of a solitary ancestor. Through individual-based simulations, we show that our mechanistic framework can reproduce colony-level characteristics of *Polistes* and, thereby, that social castes can emerge from solitary regulatory pathways. Our explanatory framework illustrates, by specific example, a changed perspective for understanding insect social evolution.

The essence of eusociality in *Polistes* wasps is the differentiation of female offspring into two castes: nonreproductive females that work in their natal colony and reproductive gynes that found colonies in the next nesting cycle (*1*). Differentiation of *Polistes* offspring into workers and gynes has been proposed to rely on physiological events that are triggered after adults emerge from pupation (*2*). A more widely held view, however, is that caste in social wasps is determined, or at least predisposed, during larval development (*3*). Larval nourishment is believed to play a role in caste differentiation (*1*, *3*), but the nature of this mechanism has not been determined. More importantly, no hypothesis addresses how a nutritional cue would translate into specific traits that characterize the two castes.

Regulatory machineries that control sequential shifts between phases in the life cycles of solitary insects may have been coopted during social evolution (*4*). From this perspective, caste evolution can be envisioned as a remodeling process that uses solitary control circuits to build social phenotypes. The regulatory layout of a presocial form would thus become the ground plan that underlies the evolutionary design of its social descendants. One such layout may be the bivoltine life cycle, an adaptation to seasonal environments that may be tropical wet/dry as well as temperate warm/cold. Bivoltine life cycles have a first generation that undergoes uninterrupted development and reproduction, followed by a second generation that enters prepupal or adult diapause, passes the unfavorable season, and reproduces the following year. (The prepupa resides inside the pupal cocoon and is a quiescent state of the last larval instar.) The biology of wasps in the vespid subfamily Eumeninae can have major explanatory implications for the evolutionary trajectories of sociality in Vespidae (*5*). Bivoltinism occurs commonly in solitary eumenines (*6*), and the eumenine-like solitary ancestor of the eusocial vespid *Polistes* may have been bivoltine. With this initial assumption, we hypothesize that the regulatory circuits that separated the two generational trajectories in the ancestor were co-opted to build a social life-history pattern in which the worker brood of *Polistes* corresponds to the first generation (G1) (Fig. 1) and the gyne brood corresponds to the second (G2) (Fig. 1).

Early-emerging *Polistes* offspring work as G1 females in a condition of reproductive readiness but in a context that curtails their reproductive contribution to the G2 brood (Fig. 2). Worker behavior in *Polistes* is context-dependent expression of maternal care behaviors (*1*), and when the females begin to forage and engage in nest construction, they do so as workers at the natal nest. The reproductive potential of *Polistes* G1 females is nonetheless readily apparent, as "laying workers" (*7*), replacement queens (*8*), and satellite nest foundresses (*9*, *10*) all come from the early brood. Thus, at the conceptual level, our approach characterizes *Polistes* offspring workers as reproductives but with their reproduction in context-dependent suppression. Newly emerged gynes may similarly be characterized as nonreproductives. Gynes do not forage or reproduce their first year, and gynes show no ovarian development or nest building behavior when isolated under favorable conditions in which G1 wasps (that normally would be workers) initiate reproductive maturation and nest construction (*11*, *12*). These data suggest that gynes emerge in a state of reproductive diapause, which is physiological arrest rather than behavioral quiescence. Developmental and adult traits that distinguish gynes from workers suggest that gynes derive from a G2 phenotype (Fig. 2).

This explanation identifies a developmental machinery that can channel individuals into two castes. Specifically, caste differentiation

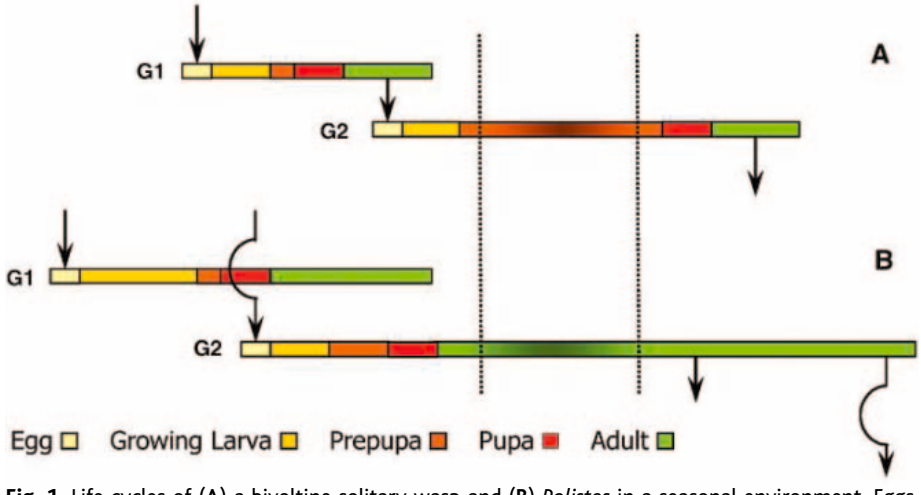

Fig. 1. Life cycles of (**A**) a bivoltine solitary wasp and (**B**) *Polistes* in a seasonal environment. Eggs of the first brood (G1) are laid by adults from the second generation of the preceding favorable season (G2). In the solitary wasp, G1 females complete development, emerge as adults, and produce the G2 generation. These individuals then pass the unfavorable season, indicated by shading between the dotted vertical lines, in prepupal diapause (*35*). Three principal changes can convert this solitary life cycle into a social life strategy. One change is that diapause is passed as an adult rather than as a prepupa, which is known to occur in some bivoltine solitary wasps in family Sphecidae (*35*). A second change is that the life cycle is partially rather than discretely bivoltine. In partial bivoltinism, adults that lay eggs of the first generation also lay some eggs of the second generation. This strategy is common and may favor evolution of eusociality (*6*). The third change is that offspring of the first generation do not reproduce but instead undertake brood care at their natal nest; thus, no arrow connects the G1 and G2 broods in the *Polistes* diagram (B).

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could rely on regulatory circuits that, in the bivoltine ancestor, controlled the conditional prepupal diapause pathway that separated the two generational trajectories (Fig. 1A). This prediction is supported by experimental evidence. The differentiation appears to be largely determined before emergence (*3*, *13*), and traits indicative of G1 and G2 phenotypes are apparent from early in life (Fig. 2). Further, the pupation time of gynes, measured from cocoon spinning to adult emergence, is longer than the pupation time of workers (*14*). Gynes also contain high levels of hexameric storage protein at the end of pupal development (*13*). These are traits normally associated with prepupal diapause (*15*). It is also worth noting that the high level of storage protein in *Polistes* gynes is not an obligatory trait in Vespidae, being absent from gynes of Vespinae (*13*). This suggests that the presence of hexamerin in *Polistes* is the signature of a specific differentiation machinery.

In solitary insects, diapause is under innate regulatory control and is characterized by specific hormonal signatures and patterns of gene expression (*15*). A social co-option of the corresponding regulatory circuits would imply that a cue stemming from the social condition now controls a modified machinery that induces a set of diapause characteristics during development and early adult life rather than triggering a state of developmental arrest. Nutrition is one factor that governs diapause initiation in solitary insects (*16*–*18*), and differential larval nutrition is a common means to induce caste-specific developmental programs in social taxa (*2*). It is also the most prevalent hypothesis for caste differentiation in *Polistes* (*1*, *3*, *13*, *14*).

To explore the validity of the assumption that a diapause switch triggered by larval nutrition is the basis of caste differentiation in *Polistes*, we examined the demographic implications by use of individual-based modeling (Fig. 3) (*19*). Each resulting pattern can be matched to examples from nature. Moderate food levels (Fig. 3A) produce dynamics that are typical for *Polistes* in seasonal environments: A peak of workers precedes a peak of gynes (*20*). Specifically, workers are individuals channeled into a G1 pathway, whereas gynes follow a G2 trajectory. Variation in nutrient inflow can translate into two distinct phenomena known in *Polistes*: a minor peak of early gynes (*21*) and a minor peak of late workers (Fig. 3B) (*22*). Higher food levels lead to early termination of brood rearing (Fig. 3C) (*23*), whereas very low food levels lead to few workers and almost no gynes (Fig. 3D) (*24*).

Summary data illustrate how the model responds to changes in average food level (Fig. 4). The level indicated by arrows is of particular interest. Here, the number of gynes has the largest variance and spans the full range for the observations (0 to 60 gynes). At the same time, the number of days until colonies reach maximum gyne production is largest. These results describe colonies that vary greatly in gyne production, with many colonies producing few gynes and a few colonies producing many gynes. High variability among colonies, with a few colonies placing large numbers of gynes into quiescence late in the nesting season, exactly characterizes annually seasonal *Polistes* populations (*1*).

The fit of the model output to actual observations in nature, both in general patterns and particular variations, suggests that a nutrient-dependent switch mechanism during larval growth is sufficient to explain caste differentiation in *Polistes*. A correlative relationship between feeding and caste determination has long been suspected (*1*, *3*), but a framework that combines a nutritional switch with a specific hypothesis on its origin has been missing. We hypothesize that *Polistes* workers and gynes derive from a regulatory ground plan: the bivoltine life cycle that was designed to produce a developmental bifurcation in an ancestral solitary form. Therefore, we not only provide a specific prediction about the developmental circuits that have been co-opted by social evolution to produce the two castes, but we also provide an explanation for the suite of traits associated with each phenotype (Fig. 2).

The transition from bivoltinism to eusociality may have occurred after the bivoltine ancestor dispersed into a favorable environment that enabled uninterrupted development of G2 females. Evolution of eusociality, however, would additionally require that G1 wasps remained as alloparental caregivers at the natal nest. Trophallactic transfer of amino acid–rich saliva (*25*) from larvae to adults may have been the key evolutionary invention that induced reproductively tuned females to remain at their natal nest (*26*). Indeed, experimental disruption of adult feeding on saliva causes social wasp colonies to fail (*24*, *27*). However, although larval saliva as an amino acid source for vitellogenesis would tie G1 individuals to the nest, the costs of foraging and nest building would constrain them from oogenesis (*28*). This scenario implies that castes in *Polistes* evolved from interactions between

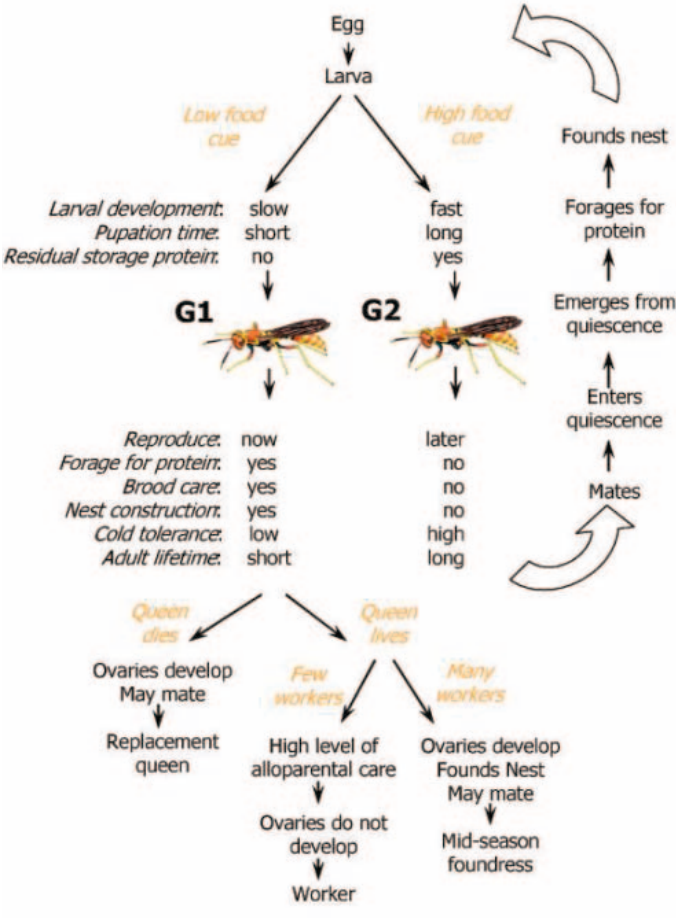

Fig. 2. The *Polistes* life cycle incorporates fundamental elements of the bivoltine ground plan. Larvae respond during development to a food cue and diverge onto one of two trajectories. Scanty provisioning leads to the G1 pathway, which is signaled by slow larval development (due to low nutrient inflow), short pupation time (*14*), and no storage protein residuum in emerging adults (*13*). More abundant provisioning leads to more rapid larval development, longer pupation time (*14*, *20*), and residual storage protein in emerging G2 adults (*13*). G1 females have a "reproduce now" phenotype, and they forage for protein, care for the brood, and construct nests. The expression of these behaviors is conditional, as indicated by branching points in the G1 sequence. If the queen is lost, a G1 female can develop her ovaries, mate if males are present, and become a replacement queen. If a queen is present but the number of workers is low, a G1 female will alloparentally express maternal behaviors (i.e., nest construction, nest defense, brood care, and foraging) as a worker at her natal nest. Finally, if a queen is present and the number of workers is high, a G1 female may depart the natal nest and found a satellite nest in midseason. Because the cold tolerance of G1 females is low, they do not survive quiescence, and lifetimes are short. In contrast, G2 females have a "reproduce later" phenotype. They express no maternal behaviors the first year, but after emerging from quiescence, they break reproductive diapause and shift to the reproduce now phenotype.

adults and larvae rather than from interactions among group-living adults, as hypothesized by West-Eberhard (*29*).

The bivoltine ground plan hypothesis has considerable explanatory power to reinterpret natural history and experimental data on *Polistes*. Foremost among these is that *Polistes* dichotomizes offspring into two behavioral categories, workers and gynes, despite an absence of morphological differences between them. These now can be seen as G1 and G2 females whose behavioral tuning reflects underlying bivoltine phenotypes. Early gynes (*21*) and late workers (*22*) show that G1 and G2 phenotype expression in *Polistes* is cued to colony conditions, which typically change in a seasonal pattern, rather than to seasonal environmental variation itself. Individual- and colony-level responses to nutrition manipulations (*14*, *24*), as well as our simulation results, support this explanation. Our framework can also be meaningfully applied to *Polistes* species that are social parasites of other *Polistes* species. The social parasites emerge from quiescence after host nesting is initiated, and they invade nests when host offspring begin to emerge. Host workers then rear all of the parasites' offspring as gynes (*30*). This suggests that the G1 generation has been deleted from the inquiline life cycle.

Specific and testable predictions derive from our framework. If the differentiation of G1 and G2 females is driven by a co-opted diapause switch, we would expect the prolonged times spent by gynes in cocoons (*14*) to be predominantly due to a longer prepupal stage. During this phase, gynes will show increased production rates of hexameric storage proteins, higher levels of accumulation compared to workers, and hormonal signatures of diapause. Further, the G1 and G2 signatures should be residual in primitively social polistine wasps living in nonseasonal tropics. This is strongly suggested by evidence from *Ropalidia marginata*, in which about half of female offspring will build nests when isolated at emergence and half will not, even though colony cycles are indeterminate and

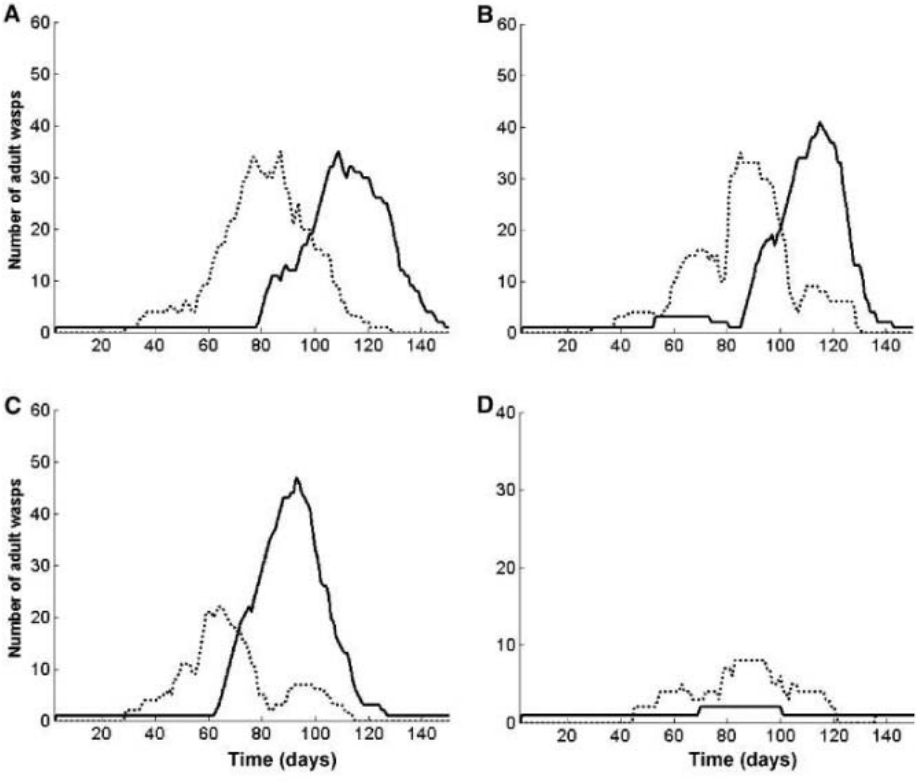

Fig. 3. Output from four separate runs illustrates the dynamics of the individual-based model. Each simulation shows the number of workers from the G1 pathway (dotted line) and gynes of the G2 trajectory (solid line) present on a nest. The amount of food available to individuals that forage is the only variable changed between runs. (**A**) Moderate food levels generate a peak of workers followed by a peak of gynes. (**B**) Day-to-day random fluctuations in food generated by the model can result in early gynes, late workers, or both in the same run. (**C**) More food leads to an earlier worker peak and to earlier production of more gynes, and the food demands of those gynes cause a late peak of workers and early termination of brood rearing. (**D**) Very low food conditions result in few offspring, almost all of which are workers, and early termination of brood rearing.

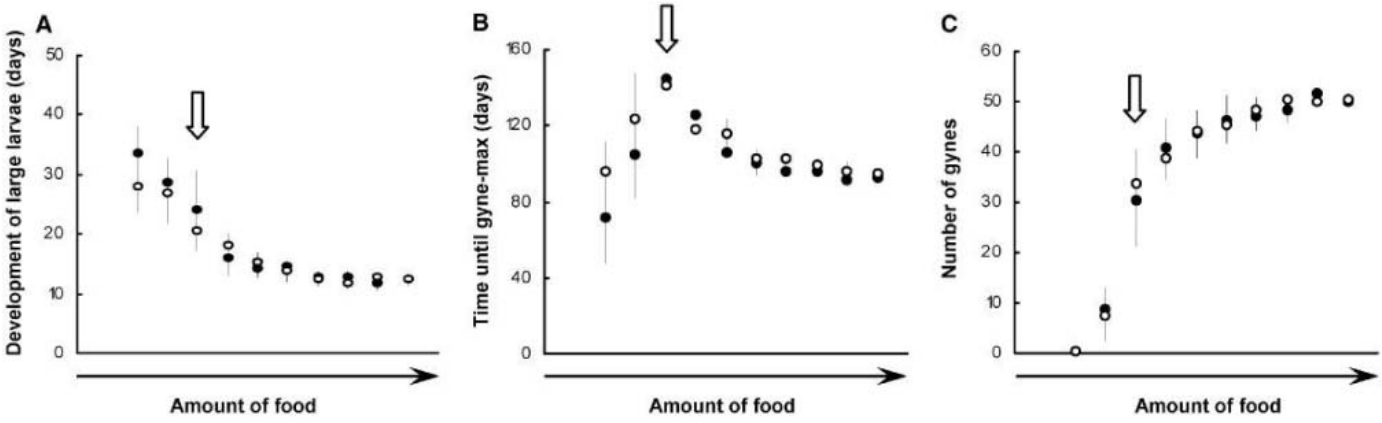

Fig. 4. Summary statistics from two sets of simulations (n = 15 runs each) illustrate both the consistency of results and the model's response to changes in average food level. Open and solid circles are the means of the two sets; vertical lines are standard errors. (**A**) The development time for large larvae is long and has a high standard error at low food levels, and both development time and standard error decrease with increasing food. In *Polistes*, larval developmental time is inversely correlated with the duration of the pupal stage (*14*), although total development time is longer for individuals that develop under low food conditions. (**B**) The time from the start of the simulation until the peak of gynes present on the nest has high variance at low levels and reaches a maximum at an intermediate food level. (**C**) The number of gynes produced increases with increasing food level. Arrows indicate the same food level, and in (B) and (C), also denote the simultaneous occurrence of the longest time until peak gyne production and the highest standard error in the number of gynes produced.

asynchronous (*31*). We further expect this form of differentiation to be absent in species that originated in a nonseasonal environment, as potentially exemplified by "facultative eusociality" in Stenogastrinae (*32*). Halictine bees may represent a separate taxon where sociality evolved from bivoltine ancestry. A prerequisite for sociality in this group is that the species is bi- or multivoltine (*33*), and the halictine literature is rich with descriptions suggestive of G1 and G2 phenotypes (*34*).

The bivoltine ground plan hypothesis of caste evolution in *Polistes* inaugurates a changed perspective on the evolution and maintenance of sociality in insects. It shifts emphasis away from altruism, away from costs and benefits, and away from conflict and cooperation. It states that evolutionary trajectories of sociality are best understood as having been shaped by regulatory circuits present in solitary ancestral forms. It calls for a mechanistic approach to caste evolution, and it illustrates, by specific example, that social evolution in insects can be fully—and finally—understood.

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The Structure of a Retinal-Forming Carotenoid Oxygenase

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Enzymes that produce retinal and related apocarotenoids constitute a sequence- and thus structure-related family, a member of which was analyzed by x-ray diffraction. This member is an oxygenase and contains an Fe^{2+}-4-His arrangement at the axis of a seven-bladed β-propeller chain fold covered by a dome formed by six large loops. The Fe^{2+} is accessible through a long nonpolar tunnel that holds a carotenoid derivative in one of the crystals. On binding, three consecutive double bonds of this carotenoid changed from a straight all-trans to a cranked cis-trans-cis conformation. The remaining trans bond is located at the dioxygen-ligated Fe^{2+} and cleaved by oxygen.

Retinal and its derivatives participate in numerous cellular activities; they are crucial for vision and the immune system (*1*, *2*) and are therefore of nutritional importance (*3*). Retinal-forming carotenoid oxygenases constitute a sequence-related family of more than 100 currently known members. The family was discovered through a 9′-*cis*-epoxycarotenoid oxygenase that participates in the biosynthesis of the important plant hormone abscisic acid (*4*). A prominent family member is β-carotene-15,15′-oxygenase from animals, which cleaves β-carotene symmetrically to two molecules of retinal (*5*–*7*). Another member is β-carotene-9′,10′-oxygenase, cleaving β-carotene asymmetrically to form apo-10′-β-carotenal (*8*), which is thought to be converted to retinoic acid (*9*), a key actor in developmental processes (*10*). The family includes the retinal pigment epithelial protein RPE65 (*11*), mutations of which cause Leber's congenital amaurosis, a severe blinding disease (*12*). In plants, the genome of *Arabidopsis thaliana* codes for as many as nine family members (*2*, *13*), several of which have been established as carotenoid oxygenases (*14*, *15*). Some of these genes yield products regulating growth and development (*16*, *17*). Other plant members catalyze the biosynthesis of pigments (*18*, *19*). Cyanobacterial retinal-producing members have been proposed (*20*) and recently identified in *Synechocystis* (*21*). Here, we report the crystal structure of the *Synechocystis* enzyme at 2.4 Å resolution, revealing the reaction geometry and establishing a solid base for modeling all other family members.

The apocarotenoid-15,15′-oxygenase (ACO) from *Synechocystis* sp. PCC 6803 was expressed in *Escherichia coli* inclusion bodies and (re)natured with yields of about 2 mg purified enzyme per liter culture (*22*). ACO was soluble without detergent. It became active after adding Fe^{2+} ions and retained its activity over several days. The catalyzed reaction requires dioxygen (Fig. 1A), but the oxygen atoms in the two resulting aldehydes most likely originate from both dioxygen and water in a 1:1 ratio (*23*). On purification, the elution pattern of the final gel filtration column showed a dominant peak at the monomer mass and a small peak at the dimer mass (fig. S1). After adding the detergent octylpolyoxyethylene (C_8E_{4-8}), the dominant peak ran at the trimer mass, presumably because ACO had been recruited to a detergent micelle. Because numerous attempts to crystallize the soluble detergent-free protein failed, detergent was added in all further experiments.

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First, inactive Fe-free ACO was crystallized in the presence of its substrate (Fig. 1) and the crystal structure was solved by x-ray diffraction (table S1). The phases were determined by two heavy-atom derivatives and solvent flattening. A model was built and refined to good-quality indices (table S2). In a second experiment, we exposed the Fe-free crystals to Fe^{2+} ions for 30 min and flash froze to 100 K. Visually, the crystals remained the same, but an x-ray analysis showed a lower symmetry space group with four independent ACO molecules instead of two in Fe-free ACO crystals (table S1). The crystal structure was solved by molecular replacement and refined (table S2). On soaking, the ACO molecules had rotated by 1° to 3° and shifted by 1 to 4 Å, improving the crystal quality. In both crystal forms, the crystallographically independent molecules associated asymmetrically, indicating that ACO is monomeric as shown by gel filtration.

During refinement, the chain folds of the four individual ACO molecules remained virtually identical to each other and also to those of the Fe-free crystals. Obviously, catalysis does not involve extended main chain displacements, because these should have been detectable in at least one of the six independently packed molecules. Fe^{2+} was bound at four histidines as derived from an 8σ electron density peak. Such an Fe^{2+} coordination is known for only four other proteins (*24*–*27*). The presence of Fe^{2+} was confirmed by an anomalous difference Fourier map showing only one significant peak.

ACO consists of a seven-bladed β propeller with four histidines at the propeller axis that hold the Fe^{2+} ion and thus mark the active center (Fig. 2). Such a chain fold was initially observed in a neuraminidase (*28*). The closest structural relative of ACO is presently a muconate-lactonizing enzyme (*29*) showing a *z* score of 17.3 in a chain-fold comparison with program DALI (*30*). Four other known structures have *z* scores above 12. The top end of the β-propeller axis as defined by the blade connections (*31*) usually accommodates the active center. This also applies for ACO. The four histidines holding Fe^{2+} are all at the beginning of the innermost β strands of the propeller (Fig. 2). Each blade consists of four locally connected antiparallel strands, except for the first and seventh blade, which are five-stranded as a result of an N-terminal addition (fig. S3). The loops at the top side of the propeller are very long, forming a large dome over the active center, whereas those at the bottom are generally short.

The surface of ACO contains a nonpolar patch that consists mostly of protruding leucines and phenylalanines (Fig. 3A). We propose that ACO uses this patch to dip into the membrane and extract its nonpolar substrate from there. In both crystal forms, two ACO molecules associate asymmetrically to form a combined nonpolar patch opposite a similar patch formed by another couple about 15 Å away. Most likely, the detergent forms micelles between these patches as known from monotopic membrane protein crystals (*32*). The high 66% solvent content of ACO crystals and the exceptional behavior during gel filtration (fig. S1) also point to this group of proteins.

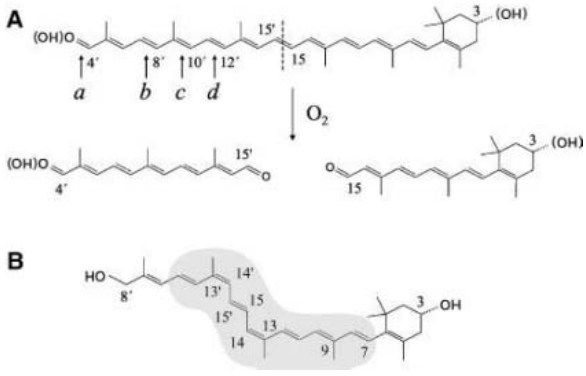

Fig. 1. Enzyme data. (**A**) The reaction catalyzed by ACO (*21*). ACO accepts the all-trans conformations of the homologs *a*, *b*, *c*, and *d* as alcohols or aldehydes with and without the 3-hydroxy group, but it does not accept β-carotene (*21*). (**B**) ACO crystallized in the absence of Fe^{2+} and in the presence of the *b*-type substrate all-*trans*-(3*R*)-3-hydroxy-8′-apo-β-carotenol. The shaded part of the substrate was used to interpret the electron density in a native crystal produced by soaking with Fe^{2+} and subsequent freezing to 100 K. Surprisingly, the all-trans substrate had changed to the 13,14-13′,14′-di-*cis* conformation.

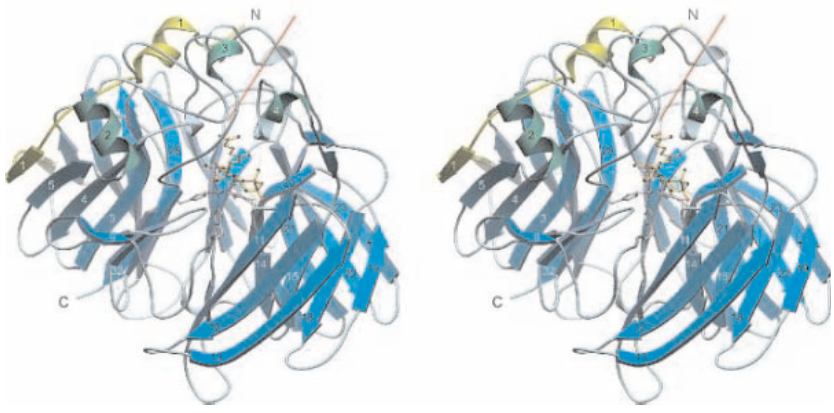

Fig. 2. Stereoview ribbon plot of the ACO chain fold consisting of 490 residues with a mass of 54,286 daltons. The 11 N-terminal residues are disordered. The seven-bladed β propeller has the usual topology (*31*). The four histidines holding the Fe^{2+} ion in an octahedral arrangement are shown together with two water molecules and the substrate as ball-and-stick models. The view is along the active center tunnel, the rear exit of which is marked by a red line. Strands β1 and β2 (yellow) are additions to the common propeller.

ACO contains a tunnel which enters the protein near the nonpolar patch and extends to the active center. After passing the Fe^{2+} ion, the tunnel turns upward and exits the protein on the far side of the nonpolar patch. This tunnel runs perpendicular to the propeller axis and is lined with numerous nonpolar residues, mainly with aromatic sidechains (Figs. 3B and 4). In addition, ACO contains a deep and narrow pocket entering from the bottom end of the propeller axis (Fig. 3B), which ends at the Fe^{2+}-ligating imidazoles and does not reach the Fe^{2+}. It results from the propeller architecture and is presumably present in all family members. In ACO, it may serve as an auxiliary pathway for dioxygen.

The inactive Fe-free crystals contained some low additional electron density in the active center that was modeled as a C_8E_4 detergent molecule. On soaking with Fe^{2+} in the presence of the substrate, however, the reconstituted native enzyme showed an electron density at the active center that was far too strong for a C_8E_4 molecule. The actual presence of a ligand was confirmed by a large rotation of the Phe^{303} sidechain providing the required space (Fig. 4). This rotation was one of the very few differences between the Fe-free and the native structure.

Because no other suitable compound had been added during crystal growth and handling (*22*), we filled this density with a substrate molecule (Fig. 4). The density had the form of a cranked rod that could only be fitted by changing the 13-14 and 13′-14′ double bonds from trans to cis (Fig. 1B). This fit implies that the β-ionone ring sits at the tunnel entrance, which acts as a bottleneck, arresting the substrate in the correct position for retinal formation (Fig. 3A). Unfortunately, because this ring is invisible, our interpretation cannot be considered proven in all details. As expected for full occupancy, the *B*-factors in the middle of the fitted substrate moiety met those of the surrounding polypeptide. They increased toward both ends, indicating that the β-ionone ring is invisible because it is mobile. At the other end of the substrate, there is space for longer isoprenoid tails, which agrees with the observed activity for 4′-apocarotenoids (*21*). β-carotene

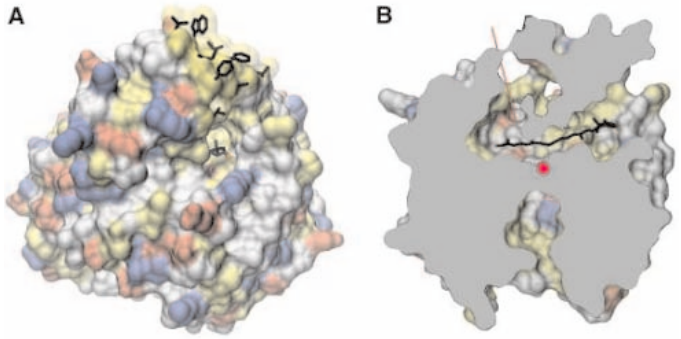

Fig. 3. Surface representation of ACO showing the general features of this putative monotopic membrane protein. The propeller axis is nearly vertical. (**A**) View into the active center tunnel, as in Fig. 2. The nonpolar patch at the top contains Trp^{121}, Ile^{125}, Phe^{126}, and Phe^{263} as well as leucines 122, 128, 259, 262, and 265 with a total nonpolar surface of about 800 Å^2. The depicted patch surface (yellow) follows fake glycines replacing the protruding residues, which in turn are shown as ball-and-stick models under the transparent true surface. In situ, this patch most likely dips into the membrane, facilitating substrate uptake and release. (**B**) Side view of the molecule as cut through the center, outlining the bent tunnel lined by the Fe^{2+} (red circle) at the active center and showing the added substrate. The small "exit" hole is indicated by the red line, as in Fig. 2. Because the deep pocket at the propeller axis at the bottom had to be cut obliquely, it shows a spurious constriction that is absent in a central cut.

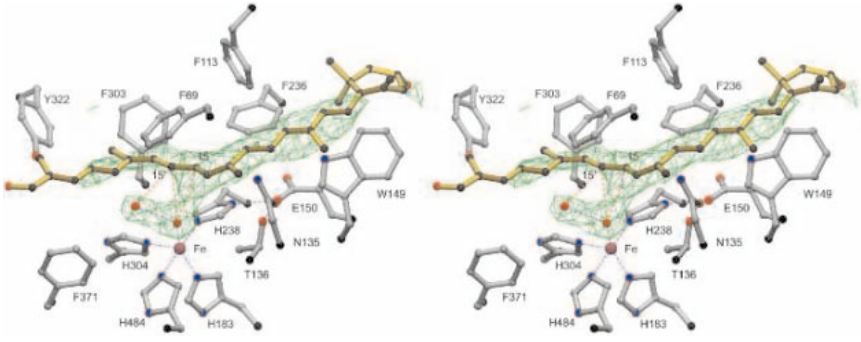

Fig. 4. Geometry of the reaction. The view corresponds to Fig. 3B. Fe^{2+} has six ligand sites arranged in an octahedron. Four sites are assumed by histidines, among which those at positions 238, 304, and 484 are fixed by glutamates. The remaining two sites most likely accept the required dioxygen. The reaction starts by one of these oxygen atoms attacking the 15-15′ double bond and continues by a further attack of a second oxygen or oxygen derivative. Because one of the produced aldehyde oxygens comes from water (*23*), it is most likely that the Fe^{2+} ligands exchange during intermediate states of catalysis. The electron densities of the substrate and water molecules represent the original (F_o-F_c)-map averaged over the four ACO molecules in the asymmetric unit at a contour level of 2σ. The double bonds of the substrate are darkened. E, Glu; F, Phe; H, His; N, Asn; T, Thr; W, Trp; Y, Tyr.

itself is not a substrate given that neither of its ionone groups can enter the tunnel. Likewise, a kinked isoprenoid tail caused by a cis double bond is not able to enter the tunnel, explaining the restriction to all-trans substrates.

When entering the tunnel, the straight isoprenoid tail collides with the oxygen ligands of the Fe^{2+} ion that most likely sterically enforce the observed two conversions from trans to cis. The isomerizations occur at methyl-substituted double bonds in an extended conjugated double-bond system with an activation energy of about 100 kJ/mol (*33*), which equals that of nonproline trans-cis peptide flips known from protein folding. The 15-15′ bond remains trans in agreement with the higher barrier of an unsubstituted double bond.

In the native structure we find a near-perfect octahedral coordination to Fe^{2+} with distances of 2.1 Å to four histidines and one water molecule. We suggest that dioxygen is bound with one atom each to the fifth and sixth Fe^{2+} coordination sites. The fifth site accommodates a water molecule in our crystal. The sixth site is large enough for one of the dioxygen atoms but unfavorable for a water because it is lined by the methyl group of Thr^{136}, which is fixed by Asn^{135} (Fig. 4). Most likely, the oxygen at the fifth site attacks the C15 atom. The distance of the respective water to the C15 atom is 3.2 Å. In our structure, we found a second water bound to the Fe^{2+}-ligated water in front of the C15′ atom. A derivative of this water may attack the C15′ atom during the reaction, explaining the observed 1:1 ratio of oxygens from dioxygen and from water in the products of a related enzyme (*23*). After double-bond cleavage, the resulting 13′,14′-*cis* and 13,14-*cis*-aldehydes readily convert to trans. Our interpretation is corroborated by the observed small amounts of 13-*cis*-retinal observed in cleavage assays (*21*).

Comparisons within the carotenoid oxygenase family (fig. S4) show that the four active center histidines are strictly conserved and that their environment is well conserved. Presumably, all members of this family share a common chain fold, possess similar active centers, and follow a similar reaction mechanism. The β propeller is a solid structural base allowing the various family members to define appropriate specificity-determining loops covering the active center. The suggested trans-to-cis isomerizations of methyl-substituted double bonds upon binding may also occur in other family members, so that some of them may be just isomerases, as is vividly discussed for RPE65 (*11*).

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AAAS Forum on Science and Technology Policy

21–22 April 2005
Loews L'Enfant Plaza Hotel
Washington, DC

Two days of federal and industrial policy-makers and members of the scientific, engineering, and academic communities participating in an open discussion of issues relating to science and technology policy.

Who should attend? Scientists and engineers, administrators, industrial R&D managers, policy-makers, academicians, association officials, federal grant recipients, students, science attachés, government affairs specialists, public affairs officers, and others with an interest in the intersection of policy with science and technology.

Included with your registration: Budget discussions will be supplemented by *AAAS Report XXX: Research and Development, FY 2006,* a comprehensive analysis of the proposals for the FY 2006 budget, prepared by AAAS and a group of its affiliated scientific, engineering, and higher education associations. Registrants will also receive *Congressional Action on R&D in the FY 2006 Budget* in the fall.

Information on registration rates, hotel reservations, and meal tickets can be found on the Web.

▸ THURSDAY, 21 APRIL

KEYNOTE
John H. Marburger, III, Director, White House Office of Science & Technology Policy

BUDGETARY AND POLICY CONTEXT FOR R&D IN FY 2006
(Plenary Session)
- Overview of Current Federal Budget Situation (David M. Walker, Comptroller General, GAO)
- AAAS Analysis of Federal Budget Proposals for R&D in FY 2006 (Kei Koizumi)
- Congressional Views of the FY 2006 Budget
- What's Happening to the Appropriations Process?
- The Federal Government: Serious or Preoccupied?

LUNCHEON AND ADDRESS
Samuel Bodman, Secretary, U.S. Department of Energy (invited)

CONCURRENT SYMPOSIA
- The Future of Scientific Communication (Formerly Known as Publishing)
- Young Scientists, Graduate Education, and National Needs for S&T Personnel
- Science and Global Health Disasters

THE WILLIAM D. CAREY LECTURE
Rush D. Holt, Member, U.S. House of Representatives (D-NJ)

RECEPTION

▸ FRIDAY, 22 APRIL

BREAKFAST AND ADDRESS
Carlos M. Gutierrez, Secretary, U.S. Department of Commerce (invited)

THE ROLE OF R&D IN THE U.S. & GLOBAL ECONOMIES
(Plenary Session)
- The View from U.S. Industry
- Josh B. Bolten, Director, White House Office of Management & Budget (invited)
- Historical View of How Technological Innovation Spurred U.S. Economic Growth
- How Does the U.S. Compare with Other Nations in R&D and Economic Development?
- How Developing Nations Are Using R&D to Build Their Economies

LUNCHEON AND ADDRESS
Speaker to be announced

SCIENCE VS. SOCIETY? WHEN SCIENTIFIC INTERESTS & PUBLIC ATTITUDES DIVERGE
(Plenary Session)

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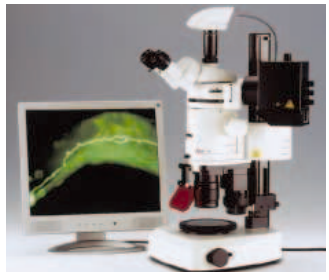

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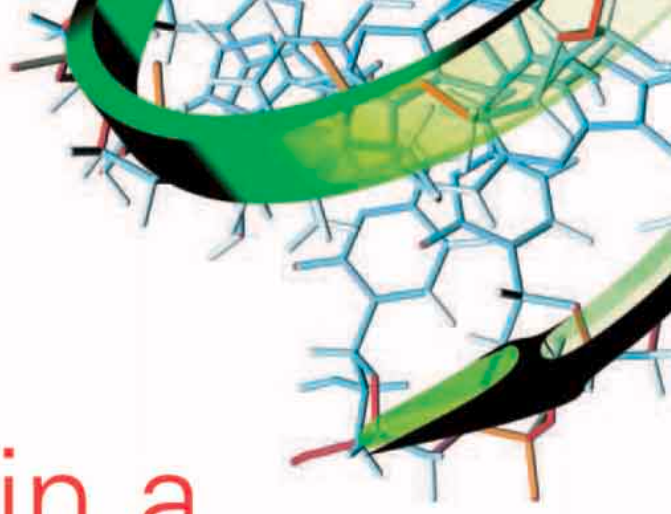

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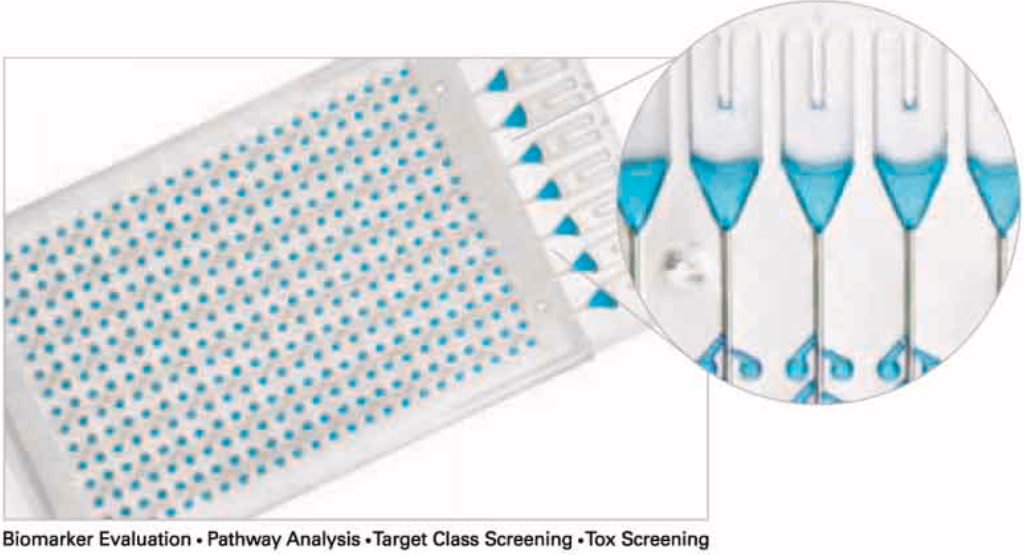

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Cancer Research

Tracking and Attacking New mechanisms to image and identify cancerous cells help scientists better understand this group of diseases. In addition, robust techniques, such as RNA interference, help researchers suppress genes involved in cancer growth. Furthermore, scientists can now control molecules that cause the immune system to kill specific cancer cells. In combination, these tools help investigators diagnose, study, and treat this deadly disease. BY MIKE MAY AND GARY HEEBNER

In this issue:

- Light microscopy
- Hyperspectral imaging
- Laser microdissection
- Angiogenesis
- Apoptosis
- TNF-related apoptosis inducing ligand
- Caspases
- Nuclear factor-κB proteins
- Kinases
- Cytokines
- Flow cytometry
- RNA interference

In many ways, today's major advances in cancer research revolve around identification and specificity. Various techniques—from labeling molecules to imaging them—help scientists identify specific kinds of cancer cells and molecules related to the progression of cancer. In addition, advancing molecular techniques make it possible to identify these cancerous components more specifically than ever.

When asked about the most important obstacles in cancer research today, Stefan Seeger, professor at the University of Zurich, Switzerland and founder of **Molecular Machines & Industries**, says, "I think the variability of cancer and the modification of it over time is a really big problem." He adds, "It's not enough to find a diagnostic marker or therapy that can be applied in general." Instead, scientists will probably need to find ways to specifically treat individuals, but that is complicated and expensive.

Seeing Is Believing

Whatever form of cancer is being studied or treated, investigators usually want to see the diseased tissue. That image often comes from light microscopy. Many manufacturers—including **Carl Zeiss**, **Leica**, **Nikon**, and **Olympus**—have been designing and producing light microscopes for both research and clinical use for many years.

Stephen Ross, senior scientist and manager of products and technology at Nikon Instruments, says that cancer research faces two technological obstacles. One is providing longterm viewing of live cells. "This requires very stable systems," Ross says, "so that scientists can do these experiments without worrying about focus drift or environmental problems in keeping cells alive for long periods." Second, Ross says that high-content screening and other techniques provide so much data that bioinformatics becomes a problem.

In late January 2005, Ross attended the Photonics West show in San Jose, California. From that experience he says, "Hyperspectral imaging was a hot topic for elucidating cancerous from healthy tissue." This technique essentially builds up an image from light across a wide range of frequencies. Ross and his colleagues recently released Nikon's first hyperspectral, confocal microscope, called the Nikon C1si.

To help investigators keep specimens in focus for long periods, Nikon also developed an autofocus technique that uses infrared MORE ›››

Inclusion of companies in this article does not indicate endorsement by either AAAS or Science, *nor is it meant to imply that their products or services are superior to those of other companies.*

» advances in: Cancer Research

light and a feedback system. Ross says, "As long as the cells' environment stays healthy, this technique can keep a structure in focus for days and days."

Getting the Right Sample

Studying cancer also depends on getting access to the right tissue or even single cells. Then, scientists can better study the process of cancer and ways to treat it. In some cases, scientists can simply order the tissue. In other cases, they must isolate it themselves.

Asterand, for example, offers human tissue that can be used in cancer research. A sample comes with clinical data, including a final pathology report in many cases. This company also makes tissue microarrays—essentially grids with tissue samples at specific *x-y* locations—that can be made from its tissue bank or other samples.

Sometimes, though, a scientist wants to obtain a microscopic sample from a specific tissue. That can be done—and under sterile conditions—with mmi CellCut, a laser microdissection instrument from Molecular Machines & Industries. With this system, a scientist can put a tissue sample under a microscope, view it on a monitor, and select an area to remove. An ultraviolet laser cuts out the sample. Also living cells can be isolated from culture after growing in a chamber. Seeger says, "You can isolate tiny compartments of even single cells from a sample with very high precision."

This technology can also be combined with molecular methods to extract DNA or RNA from a sample. "With amplification," Seeger says, "you can analyze the genome or expression profile in a single cell."

Attacking Angiogenesis

In other cases, cancer researchers want to study larger systems, such as the growth of cancer. Growth or repair of any tissue can only be sustained by building new blood vessels, which is a process called angiogenesis. Cancer cells can produce molecules that activate blood vessel formation, including vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and others. If investigators can learn to block angiogenesis in cancerous tissue, without harming the process in healthy tissue, it could be an effective treatment. Growth factors and related products can be obtained from many companies, including **Chemicon**, **ReliaTech**, **R&D Systems**, and **Sigma-Aldrich**.

Frank Mortari, director of flow cytometry and the immunohistochemistry department at R&D Systems, says, "The struggle had largely been trying to balance between the benefits and detriments of controlling vascularization of tumors." Many cancer therapies—such as chemotherapy—rely on blood vessels leading to and infiltrating tumors, but cancer researchers also want to reduce a tumor's ability to develop blood vessels that support the growth of the disease.

Richard Krzyzek, head of molecular biology at R&D Systems, says, "VEGF is one of the most potent angiogenesis factors, and we supply a large number of antibodies and recombinant proteins to VEGF family ligands and receptors." He adds, "We also make ELISA [enzyme linked immunosorbent assay] kits for measuring soluble receptors and ligands." R&D Systems also sells an ELISA kit that measures the tyrosine phosphorylation state of VEGF R2 and phosphospecific antibodies that recognize phosphorylated tyrosine sites on VEGF R2. In addition, R&D Systems recently introduced the Proteome Profiler Human Phospho-RTK Array, which can profile the tyrosine phosphorylation status of 42 different receptor tyrosine kinases, including many other receptors that are involved in angiogenesis.

Stimulating Cell Death

In development and maintenance of an organism, the death of cells is just as important as the creation of new cells. (For more on aging see the accompanying Keeping Up with Aging.) For example, programmed cell death—also known as apoptosis—plays a crucial role in the development of the brain. Some investigators also suspect that cancer might be killed if the process of apoptosis can be directed at diseased tissue. One way to do that is with tumor necrosis factor (TNF)-Related Apoptosis Inducing Ligand, or TRAIL. This transmembrane protein forms trimers that can interact with two receptors, TRAIL-R1 and TRAIL-R2, and that connection triggers apoptosis. Most important, TRAIL-stimulated apoptosis kills a broad range of tumor cells but seems to bypass most healthy cells. As a result of that, a variety of companies—including **Alexis Biochemicals**, R&D Systems, and **STI Signal Transduction Products**—offer TRAIL and related products.

Scientists at R&D Systems developed a wide range of products related to TRAIL. For instance, Mortari points out that TRAIL interacts with at least four different receptors—two that actually get activated and two decoys—and R&D Systems offers soluble versions of all four receptors and TRAIL, plus antibodies to these proteins.

Other molecules—including caspases—also participate in apoptosis. Michelle Moore, application and technical service consultant at **Roche Applied Science**, says, "There are more than 20 caspases involved in apoptosis at some point." These proteases can turn on or off steps in apoptosis. Cytokeratin 18 is one of the first molecules MORE ›››

Careers in Cancer Research

Interested in job opportunities in cancer research? A special supplement on the wide variety of careers in the field appears on page 281, following this supplement, and also runs under Career Advice on ScienceCareers.org:

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» advances in: Cancer Research

cleaved in the cascade that drives apoptosis. To help investigators track this early apoptotic event, Roche provides its M30 CytoDeath antibody. Moore states, "M30 detects the cleavage products of cytokeratin 18 in cells and tissue of tumor or epithelial origin where the cytokeratin molecule is freely expressed." In addition, this antibody can be used with flow cytometry to visualize apoptotic specific substrate cleavage. Moore adds, "The M30 antibody can also be used with paraffin embedded or frozen tissue sections. The immunoreactivity of M30 CytoDeath antibody stain is confined to the cytoplasm of the apoptotic cell. Therefore, it can be used in combination with immunohistochemical counter staining and other nuclear staining techniques."

Moore also points out that Roche makes other products that can help cancer researchers. For example, she says, "Our Cell Death Detection ELISA can differentiate between apoptosis, necrosis, and healthy cells in one assay by analyzing the relative distribution of DNA fragmentation."

Keeping Up with Aging

SAGE KE—the Science of Aging Knowledge Environment—provides a broad collection of online resources for investigators interested in the science of aging. This site features scientist written reviews, perspectives, and neurodegenerative disease case studies, as well as hot topic orientations, news synthesis articles, and weekly news stories. SAGE KE contains a genes database, tables of rodent strains used in aging-related research, a bulletin board, and a directory of scientists who study aging. This website also includes meeting information, background on funding sources, and a variety of other useful sections.

» http://sageke.sciencemag.org

Nuclear Warfare

Short of cell death, inflammation might also tell researchers something about cancer, because of nuclear factor-κB (NF-κB) proteins. Jeff Till, scientific director of phosphorylation at **Upstate**, says, "There is an apparent link between cancer and inflammation, and researchers have identified the molecular and cellular hallmarks of inflammation at the site of tumors." That made scientists wonder if the family of NF-κB proteins—all of which are transcription factors—might be related to cancer. In addition, many studies show over expression of NF-κB proteins in leukemias, lymphomas, nonsmall cell lung carcinoma, and other cancers. Recent research shows an increase in pro-apoptotic factors in the absence of NF-κB activity. Many current antitumor therapies seek to block NF-κB activity—either directly or through upstream activators, such as the I-κ-B kinase—to inhibit tumor growth or sensitize tumor cells to conventional therapies. Companies like **Active Motif**, Chemicon, and Upstate offer these products.

According to Till, when NF-κB proteins are activated, they translocate from the cytoplasm to the nucleus. So Upstate provides reagents, such as antibodies, to help scientists monitor the localization of NF-κB proteins. "The antibodies allow you to visualize NF-κB proteins translocated to the nucleus using immunocytochemistry," Till says. "Then, you can perform cell based assays with inhibitors to see if the compound prevents NF-κB proteins from getting to the nucleus."

Controlling Kinases and Cytokines

In addition to proteins, environmental cues can trigger cancer. The flow of such external signals generally gets to the machinery of a cell through kinases. The signal reaches the outside of a cell, binds to a receptor, and then a cascade of phosphorylation events—all driven by kinases—carry the signal to subcellular compartments. Kinases are offered by many companies, including Alexis Biochemicals, **Cell Signaling Technology**, **GloboZymes**, and **Stratagene**.

Chris Bunker, director, new business development at Cell Signaling Technology, says, "Many of the kinases were initially described in energy and glucose metabolism, but many more act as agents involved in cells progressing along cancerous pathways." The cancer connection arises, at least in part, because many kinases participate in growth regulation. In fact, many growth factors work through receptor tyrosine kinases. "These kinases can get turned on by point mutations or translocation events," says Bunker, "and there is no way to shut off the growth factor pathway."

Scientists at Cell Signaling Technology develop antibodies that detect phosphorylation events. Bunker says, "These can be used to see if a kinase or pathway is active in a particular type of cancer." In addition to phosphorylation-specific antibodies to monitor kinase activation, Cell Signaling Technology offers over 90 recombinant, human kinases. Bunker says, "These can be used for high throughput screening, to identify kinase inhibitors, and for lead optimization. Cell Signaling Technology has also developed the publicly accessible PhosphoSite Resource [http//:www.phosphosite.net], which provides a wealth of tools and information for kinase and protein phosphorylation research."

Many cancer researchers also explore the application of cytokines, which are hormone-like proteins made by a variety of cells. For example, lymphocytes make cytokines called interleukins. According to Xin Xiao Zheng, assistant professor of medicine at Harvard Medical School, "In cancer, cytokines play two very important roles: they can activate the immune system against a tumor, and they can also induce a tumor." Consequently, cytokines could be useful in many aspects of cancer research. For example, Terry B. Strom of Beth Israel Deaconess Medical Center and Zheng created a variety of immunoglobulin-based chimeric cytokine fusion proteins, which facilitate the study of cytokines in vivo by increasing the usually very short circulating half-lives of these proteins. **Chimerigen** licensed this technology.

As Zheng explains it, "We fuse a cytokine with the Fc, or constant, domain of an antibody. The cytokine domain of the fusion protein provides specificity." In this fused molecule, a cytokine maintains its biological function for days or weeks in vivo. The cytokine mediates immune responses to attack the cell, or tumor. In addition, the Fc domain can target the cell or tumor recognized by the cytokine moiety of the fusion proteins. MORE ›››

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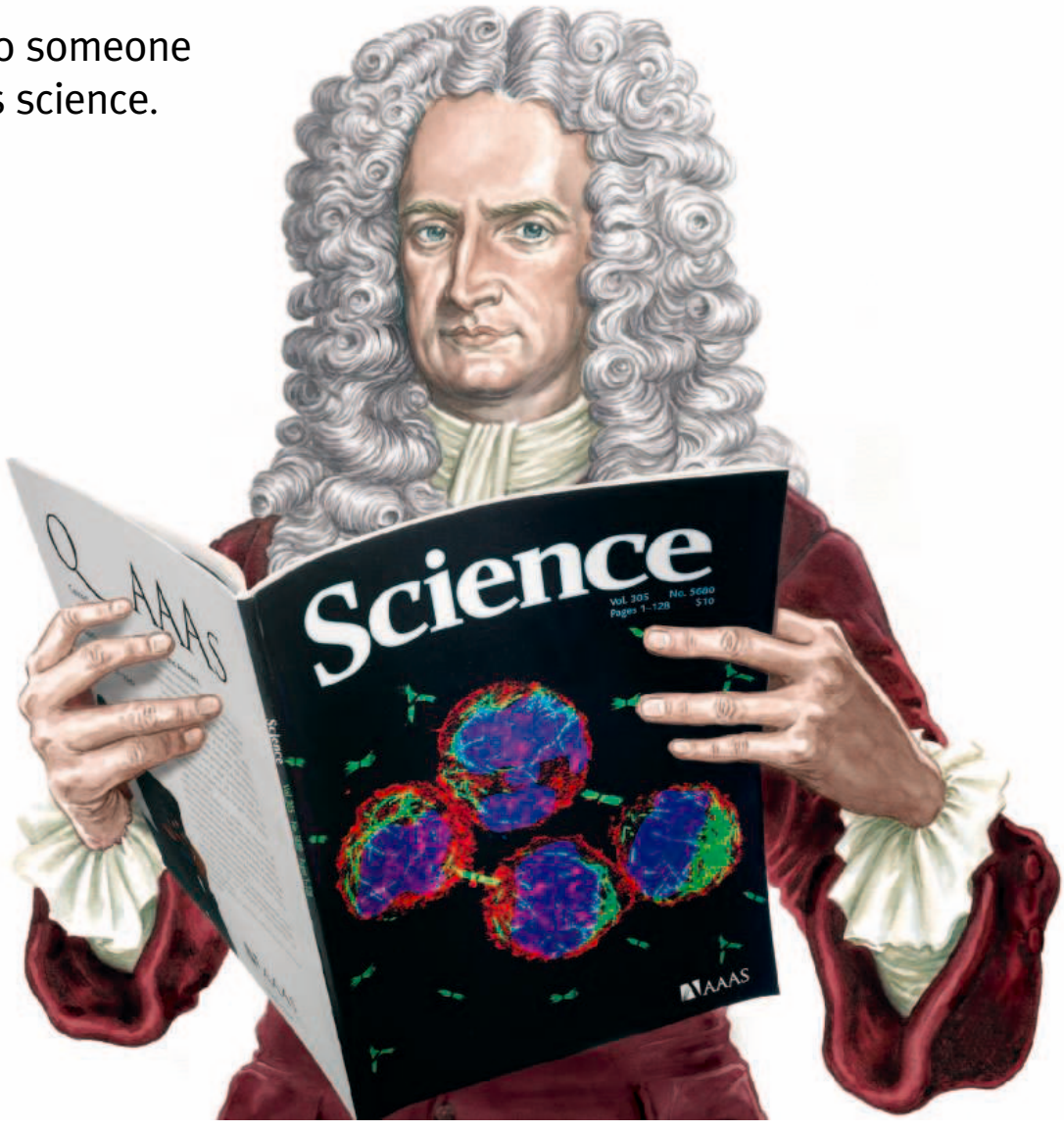

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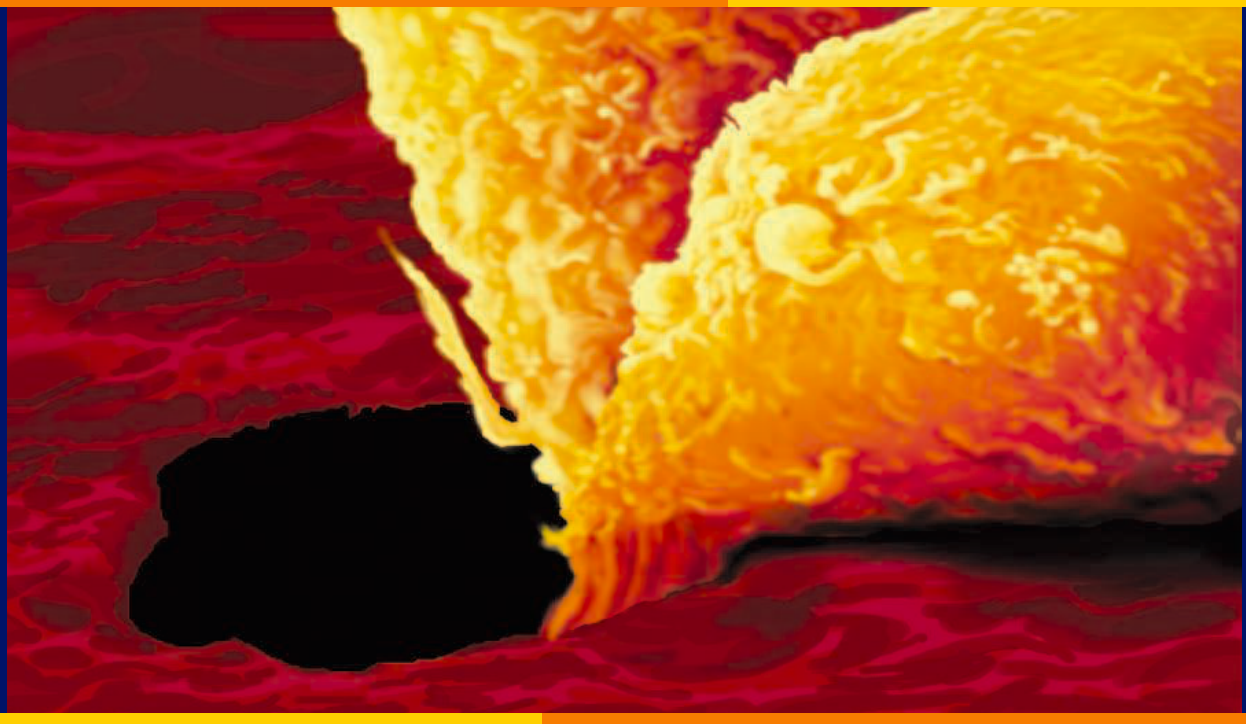

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» advances in: Cancer Research

Going with the Flow

With so many potential markers to follow, cancer researchers need high throughput techniques. One of the most reliable and fastest techniques for keeping track of cells and their function is flow cytometry. This technique can take measurements in many cells in a very short time and also profile a population of cells, based on one or more parameters of particular interest. Several companies including **BD Biosciences**, **Beckman Coulter**, and **CompuCyte** offer flow cytometers.

Kurtis R. Bray, director of research, development, and applications support at Beckman Coulter, says, "In flow cytometry, cells can be marked with antibodies or other labels, so that an investigator can perform functional and morphological analysis of cells, identify them, assess their activation state, and so on." Bray adds that there have been recent advances in automation of flow cytometry and the reagents. For example, Tandem dyes allow half a dozen colors to be followed with a single laser.

A variety of Beckman Coulter tools can be applied to cancer research. For example, Bray says, "Our CMV [cytomegalovirus] specific MHC tetramers are a really new tool to monitor one of the chief complications of stem cell transplantation, which is used to treat many lymphoid cancers." Cancer patients often become immunosuppressed during treatment, and can easily suffer reactivation of CMV. "With these MHC tetramers specific for CMV," Bray says, "you can see which patients are at higher risk of a problem and which ones aren't." He adds," We have a clinical trial under way that is determining how the CMV tetramers could be used clinically."

Running Interference

A pathway from research to the clinic could also lie ahead for RNA interference (RNAi), which can be used to suppress a gene. Jie Kang, vice president of research and development at **Qiagen**, says, "In the old days, scientists used transgenic mice to understand how a gene works. Now, you can do it much faster and easier with RNAi." She adds, "People can knock down every gene with this technique."

Kang also points out that RNAi fits perfectly with cancer research. For example, Qiagen's new HiPerFect transfection reagent works with a very low concentration of siRNA (small interfering RNA), routinely around 5 nanomolar, which is important to reduce side effects associated with RNAi. Kang says, "This reagent is quite robust with different cell lines, even difficult ones like breast cancer." In addition, Qiagen offers siRNA for the entire human, mouse, and rat genomes.

A combination of tools—from antibodies and imaging to cytokines and siRNA—could be just what researchers and clinicians need to take a more personalized approach to fighting cancer. These tactics should eventually give investigators a better idea of how cancer works, from one person to the next. For the moment, cancer researchers aim at earlier diagnosis and more specific therapies. Those goals alone provide significant challenges, as well as powerful promise.

Mike May (mikemay1@verizon.net) is a communications consultant for science and technology based in Madison, Indiana, U.S.A. Gary Heebner (gheebner@cell-associates.com) is a marketing consultant with Cell Associates in St. Louis, Missouri, U.S.A.

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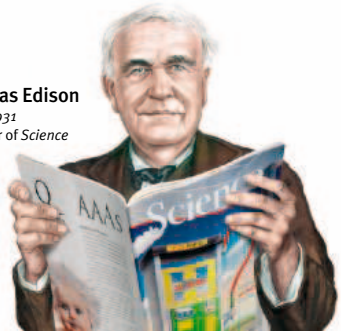

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CANCER RESEARCH

from the Bench to the Bedside

CANCER RESEARCH IS THE QUINTESSENTIAL MULTIDISCIPLINARY PURSUIT, IN THE LABORATORY AND THE CLINIC. THE TOUGHEST CHALLENGE FOR SCIENTISTS AND CLINICIANS: ENSURING A SMOOTH INTERFACE BETWEEN THEIR SEPARATE AREAS OF RESEARCH. BY PETER GWYNNE

The term "multidisciplinary" has become virtually inseparable from all facets of modern life science. But few areas demand as much understanding of a wide range of disciplines as studies of the causes of and therapies for cancer. "Cancer research is completely multidisciplinary, going from the earliest stages of investigating targets for anticancer drugs to delivering them," says David Matthews, senior director of structural biology at Exelixis, Inc. Adds Tak Mak, director of the Campbell Family Institute for Breast Cancer Research at Toronto's Princess Margaret Hospital: "It has to cover almost the whole spectrum of biological science."

John Weinstein, senior investigator at the National Cancer Institute's Center for Cancer Research, explains why. "Cancer is not restricted to one physiological system in its biology or its therapy," he says. "One has to have a rather profound understanding of multiple systems from a number of different perspectives."

In addition, cancer research involves key handoffs from scientists working in the laboratory to clinicians in the hospital. "The research needs people from many disciplines, including pure basic scientists, pharmacologists, and genomics researchers, as well as people who use clinical materials to do their studies," says William Evans, CEO of St. Jude Children's Research Hospital in Memphis, Tennessee. "Fundamental research will continue to drive the field forward. But the question is: What do you do to move it toward the bedside?"

WILLIAM EVANS

Attending Rounds

That issue obviously resonates with individual scientists. "There's greater pressure for us basic scientists to move into understanding what the clinical people are talking about," Mak says. "I regularly attend the rounds at which patients are presented." Clinicians face the same need to pick up a broad understanding of the field. "I primarily do clinical trials, but I still can't avoid multidisciplinary work," notes Melanie Thomas, assistant professor of gastrointestinal medical oncology at the M.D. Anderson Cancer Center in Houston. "Very few people here aren't going to interface with a lab in some form or other."

A broad base of knowledge is essential. "Clinicians have to understand the language of the research laboratory," Mak says. "Recently on rounds a resident discussed signal transduction." Physicians entering the field have plainly begun to realize its multidisciplinary nature. "We're seeing more and more M.D.s applying for our fellowships who have some laboratory or publication experience in their background," Thomas says. "It's pretty unusual to see someone who just wants to see patients."

Ultimately the patients are the beneficiaries of cancer research. "We regard it as our number one mission to take superlative care of patients," Thomas says. Evans agrees. "At St. Jude," he says, "the most important thing we do for patients today is giving them outstanding care; research is for tomorrow."

Cancer research involves several disciplines and subdisciplines. "Fundamental biology remains central to the foundation of the science driving cancer research," Evans says. "Genomics is increasingly important. So is epidemiology. But the rubric can also contain everything from neuroscience to stem cell research." Mak agrees. "The genomic explosion of the late 1990s will benefit cancer research more than any other field," he says. "Beyond that, from the very basic science point of view, one has to know biochemistry, cell biology, and molecular biology, and to study areas that involve growth and cell cycles, mitosis, DNA repair, and cell death."

Physics and Computer Science

The effort to discover anticancer drugs involves yet more fields. "My group at Exelixis uses physics; we rely on X-ray crystallography to look at drugs bound to target proteins," Matthews says. "There's also a huge impact from the chemistry group that synthesizes the molecules. And we have a significant computer science component in terms of both databases and molecular modeling that helps chemistry."

Clinicians who move into cancer research need to bring the basic skills common to their area of interest. "You're looking for somebody who knows how to design a rigorous clinical trial, with an under-

- **Exelixis, Inc.** http://www.exelixis.com
- **M.D. Anderson Cancer Center** http://www.mdanderson.org
- **National Cancer Institute** http://www.nci.nih.gov
- **Princess Margaret Hospital** http://www.uhn.ca/pmh
- **St. Jude Children's Research Hospital** http://www.stjude.org

Exelixis, headquartered in South San Francisco, California, is a genomics-based drug discovery company focused on the discovery and development of potential new drug therapies, specifically for cancer and other proliferative diseases. Our primary mission is to develop proprietary human therapeutics by leveraging our integrated gene-to-drug platform to increase the speed, efficiency and quality of pharmaceutical product discovery and development. Our scientists are utilizing their talents to create cures – we invite you to join them.

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  - Principal Biostatistician/Data Management
  - Senior Clinical Scientist, Development
- **DRUG DISCOVERY ADMINISTRATION**
  - Inflammation Therapeutic Area Head
- **NEW LEAD DISCOVERY**
  - Assistant/Associate Research Scientist, New Lead Discovery
- **NON-CLINICAL DEVELOPMENT**
  - Assistant/Associate Research Scientist
  - Senior Scientist, Pharmacology
- **PHARMACOLOGY**
  - Assistant/Associate Research Scientist, Pharmacology
- **PROJECT MANAGEMENT**
  - Associate Director, Project Management
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  - Associate Research Scientist

Please visit our website at www.exelixis.com to view complete job descriptions.

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Epigenetics	In Vivo Transgenic Modeling	Virology

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CANCER RESEARCH

Advances in Cancer Research
Scientists keep finding new ways to track and attack cancer, using techniques that range from antibodies and optics to cytokines and RNA interference. For more information, please see the special supplement that starts on page 273 in this issue. You can also find it in the *Science* e-marketplace:
http://www.sciencemag.org/feature/e-market/benchtop/index.shtml

standing of trial design and statistical and ethical issues," Evans says. "The clinician also has to be able to do that in the context of taking care of the patients."

A working knowledge of the impact of emerging drugs is also critical for clinicians. "Ten years ago, chemotherapy was the big way of treating cancer patients," Mak recalls. "Now, 90 percent of all drugs being developed are targeted therapeutics. There are over 800 clinical and preclinical trials going on. For those, you need to know the biomarkers to assure that the drugs are doing what they are supposed to do. Clinical correlations will be what make or break these drugs."

An essential area that applies to both laboratory and clinical studies has been neither fully recognized nor satisfied so far. "It has been predicted that by 2015 most biomedical researchers will not be doing experiments at all – they will instead be hunched over their computers poring through databases generated by biology factories," Weinstein says. "I don't believe that. But I do believe that information sciences will become an ever larger piece of the puzzle. That includes bioinformatics, biostatistics, medical informatics, and computational biology of the type involved in biological systems research. At present I feel, in particular, that most biomedical scientists and clinicians receive woefully inadequate training in statistics." As part of the effort to improve that situation, Weinstein heads an intramural program at the National Cancer Institute that aims to define the need for scientists who develop and interpret data.


DAVID MATTHEWS

Handling the Interfaces

Whatever their training, individuals involved in cancer research must quickly gain a working knowledge of the entire process. "There are challenges to address there," Matthews points out. "To some extent research scientists and clinicians speak different languages." Most important, institutions and individuals must learn how to handle the interfaces between bench scientists and clinicians, an area called translational research. "It has become more and more apparent that there has to be an active interface; it's not always easy," Weinstein says. "We have seminars and meetings at which each individual has to be a little patient as we go through background material and to agree that a certain amount of the conversation will not be of immediate interest to him or her." The M.D. Anderson Center takes the same approach. "Everybody has multidisciplinary conferences once a week," Thomas says. "We also have multidisciplinary clinics."

Weinstein's center has a somewhat ambiguous advantage that facilitates collaboration among scientists and clinicians. "We have very little space," he explains. "So people have to talk to each other. It's a big advantage in maintaining these multidisciplinary enterprises if they are located in the same space and can communicate on a daily basis."

As a drug discovery company, Exelixis handles handoffs from early in drug discovery through preclinical development and beyond. "As soon as scientists in discovery come up with an exciting project, we discuss it with our colleagues in development, looking at the promise and liabilities," Matthews explains. "A lot of the insights that our clinical colleagues might have will guide the discovery process. Once the preclinical team has started its effort, our discovery scientists are still working on the molecule to help guide design of the clinical trials. And a representative from the discovery group sits on the development project team."


MELANIE THOMAS

The need for transitions between scientists and clinicians raises administrative issues. "We make sure that our faculty evaluation and promotion system recognizes that kind of research," Evans says. "We have to recognize who is bringing unique talents to the table in translational research. As an individual, you have to know that the promotion committee recognizes your contribution to the team effort."


JOHN WEINSTEIN

Recruitment Needs

There is no lack of demand for recruits to cancer research programs. "We are recruiting scientists and clinicians all the time," Evans says. "It's a lot of my job." Adds Thomas: "We're always looking for people who can really add to the group." Mak outlines some specific needs for the Campbell Family Institute for Breast Cancer Research. "We are recruiting two clinicians and four basic and translation scientists," he says. "The clinicians will coordinate clinical trials, and for the scientists we have programs looking at genetic aberrations." Weinstein also has a wide range of needs. "We do both the experimental work and the theoretical studies," he says. "So I look for experimentalists, statisticians, bioinformaticists, and software engineers to turn our output into molecular information and computer tools that the entire cancer community can use."

Beyond top-notch scientific ability, recruiters look mainly for evidence of collegiality and communication skills. "We seek somebody who will be excited rather than intimidated by the challenge of working with these diverse groups of people," Matthews says. "You have to be able to work with surgeons, work with specialists in various disciplines, and work with people outside the institution," Thomas says. "Quality of life in the laboratory is important both for its own sake and for scientific productivity," Weinstein adds. "We need people who will perform reliably and whose work can be trusted in both the technical and ethical senses. And I treasure people to whom I can delegate with confidence."

Visit **www.sciencecareers.org** and plan to attend upcoming meetings and job fairs that will help further your career.

A former science editor of Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

Massey Cancer Center
Molecular Radiobiology

The Department of Radiation Oncology at Virginia Commonwealth University is offering a tenure-track position at the level of Assistant or Associate Professor in its Molecular Radiobiology Division. The Department of Radiation Oncology, a component of the NCI-designated Massey Cancer Center, supports a highly integrated program studying cellular radiation responses spanning radiation-induced protein modifications, signal transduction responses and DNA damage processing funded by R01 and P01 grants from the NCI (**www.radonc.vcu.edu**). The successful candidate's research interests should complement existing expertise and he/she should be interested in collaborative research with other members of the Division. The Division is well-equipped for modern cell and molecular biology studies with translational emphasis including an ABI QStarXL mass spectrometer for protein sequencing and deconvolution fluorescence microscopy for 3D image analysis. Cancer center-sponsored core facilities for virus production, flow cytometry, DNA sequencing and synthesis, and structural biology (x-ray crystallography and NMR) are also available. Support will be provided for at least three years after which extramural research funding is expected. Qualifications: A Ph.D., M.D., or MD/Ph.D. with at least 3 years of post-doctoral research experience. To qualify for Associate Professor level position, extra-mural (NIH or similar) funding is required with significant peer-reviewed publications in his/her field.

Interested candidates should submit a CV, detailed research plan and three references to:

Susan Kelly, Search Committee Administrator
VCU Department of Radiation Oncology
P.O. Box 980058
Richmond, VA 23298-0058
email: swkelly@vcu.edu
Fax: (804) 828-6042

VCU is an EEO/AA Employer. Women, minorities, and persons with disabilities are encouraged to apply.

Senior Research Scientist, Advanced Therapeutics

Vancouver, British Columbia, Canada

Named one of the world's most livable cities, Vancouver (http://www.city.vancouver.bc.ca/visitors.htm) enjoys a mild west coast climate, year-round outdoor activities, an active cultural scene, and an array of fine and ethnically diverse restaurants.

The British Columbia Cancer Agency (BCCA) has opened the doors to a new Research Centre with state-of-the-art genomic, proteomic, tissue micro-array and animal research facilities.

You will join an established, expanding translational cancer research centre engaged in pre-clinical and clinical developments of novel strategies for the treatment of cancer. You will be called upon to develop an externally funded research program to complement existing research strengths as well as participate in the design/development of preclinical programs to support clinical developments.

An accomplished Biochemist, you bring a PhD or MD; an outstanding record of achievement, including publications and grants; and an active interest in the identification/assessments of existing and novel therapeutics.

Full details of the responsibilities and qualifications are available on our websites (addresses appear below). Please forward a CV, a statement of research interests and accomplishments, and the contact information for 3 referees, by April 22, 2005, to: Mr. Chris Simms, Programs Manager, Department of Advanced Therapeutics, BC Cancer Agency; email: csimms@bccancer.bc.ca.

Those currently legally entitled to work in Canada will be given first consideration.

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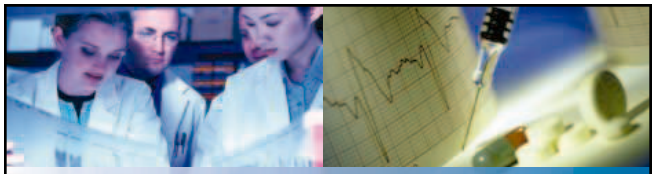

NATIONAL CANCER INSTITUTE
FOOD & DRUG ADMINISTRATION

Research & Regulatory Review Fellowships

The National Cancer Institute (NCI) and the Food & Drug Administration (FDA) are providing Research and Regulatory Review Fellowship programs to train a cadre of scientists in research and research-related regulatory review so that they can develop skill sets that bridge the two distinct processes.

The NCI-FDA fellowships offer an unprecedented career opportunity for participating researchers to become uniquely positioned to facilitate the new age of molecular medicine. Fellowships are available in Clinical Oncology Product Research/Review and Cancer Prevention.

Benefits to Researchers

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Eligibility Requirements

- M.D. and/or Ph.D., or equivalent doctoral degree
- U.S. citizenship or permanent residency
- Other requirements as specified for each fellowship program

More Information

Visit the NCI-FDA Research and Regulatory Review Fellowships Web site at ***http://iotftraining.nci.nih.gov*** for additional information on program lengths, eligibility requirements, and curricula. Or contact:

Oncology Product Research / Review Fellowships
CCR Office of Training and Education
CCR Office of the Director
National Cancer Institute
Building 31, Room 4A48
31 Center Drive
Bethesda, MD 20852
Tel: 301-451-9638
Email: wiestj@mail.nih.gov
Web site: http://iotftraining.nci.nih.go v

Cancer Prevention Fellowship
Division of Cancer Prevention
National Cancer Institute
6130 Executive Blvd
EPN, Suite 3109
Bethesda, MD 20892
Tel: 301-496-8640
Email: cpfpcoordinator@mail.nih.gov
Web site: http://cancer.gov/prevention/pob

The Children's Medical Research Institute and the Department of Pediatrics at the University of Oklahoma Health Sciences Center Announce….The Childhood Cancer Research Program to include newly developed CMRI Endowed Chairs in Pediatric Hematology/Oncology Research

DEPARTMENT OF PEDIATRICS
The University of Oklahoma
HEALTH SCIENCES CENTER

The Section of Pediatric Hematology/Oncology at the University of Oklahoma Health Sciences Center invites applications/nominations for faculty at the Associate Professor/Professor level to fill at least four positions for the newly created CMRI Endowed Pediatric Cancer Program. Program leader/s will be nationally recognized investigator/s (MD or MD/PhD – BC or BE in pediatrics and pediatric hematology/oncology) with clearly demonstrated investigative accomplishments and the desire to direct a nationally competitive research program and endowed research positions. Ample funds are allocated for startup funding. These positions may be either MD or PhD laboratory based investigators. All of these endowed chair positions will obtain and sustain independent, external grant funding.

The research program will be an integral part of the OU Cancer Center, supported in part by an NCI Comprehensive Cancer Center planning grant (P20). A new research building will open in 2005 that will contain cancer and genetics researchers.

The Section of Pediatric Hematology/Oncology presently comprises seven faculty with diverse clinical, investigative interests and includes an active oncology program that accrues more than 70 new cancer patients yearly, a federally funded Center for Bleeding Disorders, and is part of the Comprehensive Sickle Cell Center of the Southwest, one of 10 federally funded sickle cell centers in the U.S. Our bone marrow transplant center is an approved COG transplant institution and is part of the National Marrow Donor Program. The outpatient center will move to a new Children's Ambulatory Center in 2007.

The University of Oklahoma Health Sciences Center includes seven colleges and 17 other institutions and serves as our state's principal medical education and research facility. The Department of Pediatrics is a multifaceted department with 16 sections and more than 90 faculty members. Clinical services are based at The Children's Hospital @ OU Medical Center, the only comprehensive tertiary and quaternary health care facility in the state dedicated to the care of children. The Children's Hospital will occupy newly renovated space in 2006.

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CMRI Endowed Chairs

1994	Terrence L. Stull	CMRI Patricia Price Browne Distinguished Chair
1997	James A. Royall	C.R. Anthony Chair in Pulmonology
1997	William H. Meyer	Ben Johnson Chair in Hematology/Oncology
1998	John J. Mulvihill	Kimberly V. Talley Chair in Genetics
1999	Kenneth C. Copeland	Ruth & Paul Jonas Chair in Endo/Diabetes
2000	Marilyn B. Escobedo	Reba McEntire Chair in Neonatology
2001	Mark L. Wolraich	Shaun Walters Chair in Dev & Behav Pediatrics
2002	David W. Tuggle	Paula Milburn Miller Chair in Pediatric Surgery
2003	Martin M. Turman	Wal-Mart/Sam's Club Chair in Nephrology
2003	Joan Cain	Endowed Chair in Pediatric Education

Interested applicants should submit a letter of interest, curriculum vitae, and the names of three references to:
William H. Meyer, M.D. (attention: Brenda Freese)
CMRI Ben Johnson Chair in Hematology/Oncology
The Children's Hospital @ OU Medical Center
940 NE 13th St., Room 2B2308
Oklahoma City, OK 73104 e-mail: william-meyer@ouhsc.edu

MEDICAL COLLEGE OF WISCONSIN

Cancer Research Positions

The Cancer Center of the Medical College of Wisconsin is actively expanding its scope and size. Successful candidates are expected to assume leadership roles and help develop cancer research programs. Development funds, core support, and traditional-track appointments are available for each position.

Breast Cancer Research	Molecular Genetics of Oncogenesis	Biochemistry	Pharmacology and Toxicology	Pathology
Laboratory-based or translational research Focus on genetic mechanisms in breast cancer desired Eligible for **Joan A. Van Deuren Professorship in Breast Cancer Research**	Assistant/Associate Professor Positions Genomic Instability DNA Repair Cell cycle control and checkpoints Join an active and collegial research-oriented department	Assistant/Associate Professor Positions Signal Transduction Structural Biology Proteomics Join an active, well-funded department	Assistant/Associate Professor Positions Signal Transduction Angiogenesis Growth Factors	**Chair of Clinical Department** Senior leadership skills and national recognition within academic pathology Commitment to scholarship, education, research and clinical service It is essential that the candidate develop cancer-related basic research within the department and in collaboration with other departments The department has a solid financial base with multiple opportunities for clinical income
William Campbell, PhD Chairman, Pharmacology & Cancer Center Recruitment Committee **414/456-8267** **wbcamp@mcw.edu**	**Paula Traktman, PhD** Chairman, Microbiology and Molecular Genetics **414/456-8253** **ptrakt@mcw.edu**	**Robert Deschenes, PhD** Chairman, Biochemistry **414/456-8435** **rdeschen@mcw.edu**	**William Campbell, PhD** Chairman, Pharmacology and Toxicology **414/456-8267** **ptsearch@mcw.edu**	**Dale K. Heuer, MD** Chair, Pathology Search Committee Chairman, Ophthalmology **414/456-7915** **dheuer@mcw.edu**

Clinician Scientists — Concurrent with the above recruitments, MCW is actively seeking MD and MD/PhD clinicians specializing in Medical Oncology and Surgical Oncology.

To apply, send CV to contact listed above at: **The Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226**. For more information about MCW, visit our website (**www.mcw.edu/cancercenter**), or **Contact Bruce H. Campbell, MD, FACS, Interim Director of the Cancer Center 414/805-4455** or **bcampbel@mcw.edu**.

EOE M/F/D/V

CAREERS IN CANCER RESEARCH

Fujirebio, Inc. is seeking a Post-Doctoral Research Fellow (RF) in the field of Microbiology/ Molecular Biology/Biochemistry

The new RF will join a Microbial Metabolic Engineering project at the multi-national Frontier Research Department of Fujirebio Inc. that investigates the metabolic flow through biochemical pathways and its influence on the bacterial synthesis of H_2.

The ideal applicant is looking for a first or second post-doc and is hungry to produce high-quality high impact papers to advance their career. Applicants with experience in microbiology (esp. Archaea), fluxome analysis or metabolic pathway modeling are particularly encouraged to apply. A background that includes molecular biology techniques is essential.

Contract-based hiring: Minimum length of 2 years, competitive salary. Research Location: Fujirebio Research facilities located in the city of Hachioji, western Tokyo, Japan. The modern, spacious and well-equipped laboratory is located close to mountains (incl. Mt. Fuji) and national parks and is only 40 minutes away (by train) from central Tokyo.

Interested applicants should send a detailed CV along with at least two academic references.

Contact:
Fujirebio.Inc, Frontier Research Department, Dr. Masahisa Okada, 51 Komiyacho, Hachioji, Tokyo, 192-0031, Japan.
Tel: +81 (426) 45-4740,
Fax: +81 (426) 46-8325,
E-mail: ms-okada@fujirebio.co.jp

CANCER PATHOBIOLOGY RESEARCH FACULTY POSITIONS UNIVERSITY OF MISSOURI - COLUMBIA

The University of Missouri invites applications for two tenured or tenure-track faculty positions in cancer pathobiology, one at an assistant to associate professor level and one at the associate to full professor level. The University of Missouri-Columbia is noted for interdisciplinary research programs that have been enhanced recently by the opening of the $60 million Life Sciences Center.

Applicants for the positions would be expected to have or develop an independent research program in cancer pathobiology, and to become involved in campus-wide programs in cancer, epigenetic mechanisms of gene regulation, proteomics, models of disease in alternative species, and/or reproductive/developmental biology.

Applicants should have a strong interest in contributing to graduate and medical education in cancer biology, pathology, and anatomical sciences. The position may include a leadership role in the cancer center research efforts of the Department of Pathology and Anatomical Sciences in the School of Medicine.

Interested individuals should submit a letter of interest, current CV and a list of three references to:

Douglas C. Anthony, M.D., Ph.D.
Professor and Chair
Department of Pathology and Anatomical Sciences
M263 Medical Sciences Building-DC055.07
University of Missouri School of Medicine
One Hospital Drive
Columbia, MO 65212

or by electronic submission (preferred) to:
nicholsrkn@health.missouri.edu

Review of applications will begin in **April 2005**, and will continue until the position is filled.

The University of Missouri is an Equal Opportunity Employer, Affirmative Action Employer, and complies with the guidelines set forth in the Americans with Disabilities Act of 1990. Applications from members of underrepresented groups are encouraged. For ADA accommodations, please contact our ADA coordinator at (573) 884-7278 (V/TTY).

CAREERS IN CANCER RESEARCH

The **Institute for Cancer and Stem Cell Biology and Medicine** at **Stanford University** is holding an open search for **tenure-line faculty** in the area of cancer gene discovery. The faculty slots can be at the assistant, associate or full professor level.

Successful candidates will have an outstanding record of research in cancer gene discovery and a strong interest to help translate these discoveries to pre-clinical research and potential therapies. A strong focus of the Institute group is the discovery of cancer and leukemia stem cells, and new faculty would be expected to interact with the established Cancer/Stem Cell group to help elucidate steps in the progression from normal cells to frankly malignant cells. Stanford has established a Comprehensive Cancer Center, and cancer gene discovery will be a focus for the Cancer Center.

The most successful candidates will have defined high through-put methods to discover activated proto-oncogenes resulting from genetic or epigenetic regulation as well as inactivated tumor suppressor genes. Relevant examples would be outstanding mouse geneticists with an exceptional record of research who use, and are developing, novel technologies and genetics approaches that will lead to the better understanding and treatment of cancer and other human genetic diseases.

All appointments to the Institute and the Cancer Center will be in departments at Stanford University, so interested candidates should indicate which departments at Stanford would be among their first choices for department affiliation.

The appointees will be in laboratories in the Institute, and will participate in Institute research and teaching activities. While teaching is an important endeavor at Stanford University, these appointments (Institute for Cancer and Stem Cell Biology and Medicine appointments) will be primarily based on research accomplishments and the promise of future research advances.

Salary will be commensurate with the level of employment, relevant experience and accomplishments. Please send letters of application, including full curriculum vitae and the name and addresses of three references to: **Randy Mont-Reynaud for I. L. Weissman, Director of the Institute for Cancer and Stem Cell Biology and Medicine, Room L322, Stanford University School of Medicine, Stanford, CA 94305-5324**.

Stanford University is an Equal Opportunity, Affirmative Action Employer.

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

National Human Genome Research Institute

Program Director, Mouse Genomics
Program Director, Medical Sequencing

The Division of Extramural Research of the National Human Genome Research Institute (NHGRI) seeks candidates for two Program Director positions as the Institute expands its activities in several areas of genomic research, specifically mouse genomics and medical sequencing.

Program Director, Mouse Genomics: The NHGRI is co-leading the development of a proposal for a trans-NIH initiative to develop a new mutant mouse resource that would improve the utility of the mouse as a model for the study of human disease and biology. This resource will comprise a comprehensive set of mouse ES cells with null mutations in the entire set of protein-coding genes. This is the first phase of the Mouse Knockout Project, described in the commentary published in Nature Genetics (9:921-924 (2004)). The Institute is searching for a highly qualified scientist with extensive experience in mouse genetics to lead the NHGRI's participation in the planning, implementation and management of this initiative, and of the portfolio of research grants, cooperative agreements and/or contracts by which the initiative will be implemented. Knowledge of mouse genetics and genomics (preferably including experience with knockout technology), and the use of bioinformatics tools is necessary.

Program Director, Medical Sequencing: With the completion of the human genome sequence, and the acquisition of the genomic sequences of a number of other organisms for the purpose of aiding the annotation of the human sequence, NHGRI now intends to direct its large-scale sequencing program toward the long-range objective of making DNA sequencing a tool for both research and medical practice. The Institute is searching for a highly qualified scientist with experience and interest in the further development of large-scale genomic sequencing and its applications in biomedical science to contribute to the continuing development and management of the NHGRI's large-scale sequencing program. Knowledge of genomic sequencing and genomic analysis (particularly as applied to problems in translational research and medicine), and the use of bioinformatics tools is necessary.

Responsibilities for both Program Director positions will include the development and implementation of new program initiatives, administration of a portfolio of research awards, and interaction with researchers and related programs at NHGRI, NIH and other research funding agencies (both public and private, in the U.S. and abroad). For each position, candidates must have an M.D., Ph.D., or equivalent-level degree and should have considerable research experience in the appropriate field. Preference will be given to candidates with experience in research management. The ability to work both independently and collaboratively as needed is essential, as are strong communication, writing, and organizational skills. The positions may be filled on a permanent or rotating basis. Salary will be commensurate with experience. Send CV, bibliography and the names of 4 references by email to **nhgrijobs-r@mail.nih.gov or FAX: (301) 480-2770. Applications will be considered every two weeks until May 30, 2005.**

National Human Genome Research Institute

Program Director, Computational Biology
Program Director, Cheminformatics (Roadmap)

The Division of Extramural Research of the National Human Genome Research Institute (NHGRI) seeks candidates for two Program Director positions as the Institute expands its activities in several areas of genomic research, specifically bioinformatics and cheminformatics. The latter position is related to the NIH Roadmap for Biomedical Research.

Program Director, Computational Biology: Bioinformatics and computational biology, including the areas of data management, storage, distribution and analysis, lie at the heart of genomic research. The NHGRI portfolio in this area includes the support of critical model organisms and other databases, algorithm development in the area of genomics, and the computational component of several state-of-the-art development programs, including ENCODE, the Knockout Mouse Project, and medical sequencing. The Institute is searching for a highly qualified scientist with experience and interest in the further development of computational biology and bioinformatics and their application in biomedical science to contribute to the continuing development and management of the NHGRI's computational biology program. Knowledge of the development and use of bioinformatics tools (particularly as applied to problems in translational research and medicine), and genomic analysis is necessary.

Program Director, Cheminformatics: This position is related to the NIH Roadmap for Biomedical Research. The NIH Roadmap is a trans-NIH effort to identify major new research opportunities and gaps in biomedical research that no single institute at NIH could tackle alone, but that the agency as a whole must address, to make the biggest impact on the progress of medical research. Within the Roadmap, the Molecular Libraries Initiative will develop a robust public sector program that will give biomedical researchers access to new small organic molecules that can be used as chemical probes to study the functions of genes, cells, and biochemical pathways.

One component of the Molecular Libraries Initiative is a Cheminformatics research program. NHGRI is seeking an exceptionally qualified informatics or computational scientist to serve as Program Director, Cheminformatics. The successful applicant will develop and manage a portfolio of research grants in cutting-edge areas of cheminformatics, and will also participate in the development and implementation of informatics strategy and policy for the Molecular Libraries Initiative.

Responsibilities for both Program Director positions will include the development and implementation of new program initiatives, administration of a portfolio of research awards, and interaction with researchers and related programs at NHGRI, NIH and other research funding agencies (both public and private, in the U.S. and abroad). For each position, candidates must have an M.D., Ph.D., or equivalent-level degree and should have considerable research experience in the appropriate field. Preference will be given to candidates with experience in research management. The ability to work both independently and collaboratively as needed is essential, as are strong communication, writing, and organizational skills. The positions may be filled on a permanent or rotating basis. Salary will be commensurate with experience. Send CV, bibliography and the names of 4 references by email to **nhgrijobs-r@mail.nih.gov or FAX: (301) 480-2770. Applications will be considered every two weeks until May 30, 2005.**

WWW.NIH.GOV

National Heart, Lung, and Blood Institute

Postdoctoral Fellowship
Chromatin and Gene Expression

Dr. Keji Zhao's lab in the Laboratory of Molecular Immunology is recruiting a postdoctoral researcher to study the function and regulation of chromatin structure. Exciting projects include (1) the mechanisms of cancer formation by mis-regulation of chromatin structure, (2) regulation of chromatin structure by Z-DNA, (3) the function of the mammalian SWI/SNF-like chromatin remodeling complexes in cellular antiviral activities, (4) development of novel genome-wide mapping techniques for identification of chromatin modifications and transcription factor target sites. (For recent publications, please see: Cell, vol 106, 309-318; Mol. Cell. Biol., vol 24, 4476-4486; Nature Biotechnology, vol 22, 1013-1016; Genes & Development, vol 19, 542-552.) We will use both in vitro and in vivo methods including knockout mice to address the questions. The successful candidate should have a Ph.D. and/or M.D. with strong background in biochemistry or molecular and cell biology. The candidate will be supported with an excellent intramural NIH fellowship in a stimulating and interactive research environment at NIH.

Send CV and names and addresses of three references to: **Keji Zhao, Ph.D., Investigator, Laboratory of Molecular Immunology, NHLBI, NIH, Bldg.10, Rm.7N311, 9000 Rockville Pike, Bethesda, MD 20892-1674, E-mail: zhaok@nhlbi.nih.gov, Fax: 301-480-0961. Application deadline: June 30, 2005.**

National Heart, Lung, and Blood Institute

Postdoctoral Fellowship
Laboratory of Molecular Cardiology

The Developmental Biology Center, Laboratory of Molecular Cardiology, is seeking a postdoctoral fellow who has obtained an M.D./Ph.D. within the past 2 years. This postdoctoral fellow will join an active program studying the role of nonmuscle myosin IIs in mouse and human embryonic development. Previous experience in developmental biology, cell biology, and molecular biology is desirable. The successful candidate will have a number of core facilities (microscope, proteomic, imaging, transgenic, etc.) at their disposal and will be encouraged to develop their own approaches to understanding the role of nonmuscle myosins in mouse and human development and disease processes.

For more information, **please consult our web page and PubMed or e-mail Dr. Robert S. Adelstein at: AdelsteR@nhlbi.nih.gov**. Applicants should submit their C.V. and arrange for three letters of recommendation to be sent either to the above e-mail address or to: **Dr. Robert S. Adelstein, Laboratory of Molecular Cardiology, NHLBI/NIH, Building 10, Room 8N202, 10 Center Dr, MSC 1762, Bethesda, MD 20892-1762.**

Applications should be submitted by May 31, 2005.

National Heart, Lung and Blood Institute

Postdoctoral Positions in
Molecular Physiology & Proteomics of the Kidney

Postdoctoral positions are available in the Laboratory of Kidney & Electrolyte Metabolism at the National Institutes of Health, Bethesda, MD (starting 7/05 or 1/06). Applicants should have either a PhD or MD degree and less than four years of postdoctoral experience. One position is for an individual to use transgenic technology in mice to investigate regulation of the water channel aquaporin-2 in renal collecting duct. Experience in basic molecular biological techniques is required and a fundamental understanding of transport physiology is highly desirable. NHLBI has an outstanding Transgenic Core Facility that can be exploited for these studies. A second position is for an individual to use mass spectrometry-based proteomics methodologies to investigate signalling pathways associated with the actions of vasopressin in the renal collecting duct. Experience in basic techniques of protein chemistry is required and a fundamental understanding of kidney physiology is highly desirable. NHLBI has an outstanding Proteomics Core Facility that can be exploited in these studies. For more information, see: **http://dir.nhlbi.nih.gov/labs/lkem/rm/index.asp**

Appointment and salary are dependent on experience. Applicants should submit a letter of interest, curriculum vitae, and the names of three individuals willing to provide letters of reference to: **Mark A. Knepper, MD, PhD, Chief, Laboratory of Kidney and Electrolyte Metabolism, Building 10, Room 6N260, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892-1603.**

Applications should be submitted as soon as possible, but no later than June 1, 2005.

National Heart, Lung, and Blood Institute

Postdoctoral Fellowship
Cardiovascular Branch

The National Heart, Lung and Blood Institute (NHLBI), Division of Intramural Research seeks, to hire a Postdoctoral Fellow to work within a Molecular Biology Laboratory in the Cardiovascular Branch. Area of focus is the molecular regulation of mitochondria in cardiovascular health and disease. Research work will use various genetic approaches in animal disease models and/or in cell culture.

The postdoctoral position requires a Ph.D. and/or M.D. degree. Applicants should have experience in molecular biology and a history of prior publications in peer reviewed journals. The successful candidate will be offered stipend support commensurate with experience.

Please submit a *curriculum vitae*, a brief statement of future research interests and future career goals, along with the names and telephone numbers, postal and e-mail addresses of three references by June 1, 2005 to: **Michael N. Sack, M.D., Ph.D., Investigator –Molecular Regulation of Mitochondrial Biogenesis and Metabolism. Email: sackm@nhlbi.nih.gov**

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Drug Discovery Senior Scientist/Principal Scientist–Oncology

Spring House, PA (Req. 0504074)

Candidates should have 4–10 years research experience in Oncology Research, after receipt of doctoral degree. Possible therapeutic strategies might include targeted therapies focused on regulatory or growth factor kinases, angiogenesis, stress response pathways, ubiquitination pathways, or the role the proteosome in cancer cell survival. In vivo experience is desirable. PhD in Molecular/Cellular Biology, Biochemistry, Pharmacology, or Cancer Biology with 4–10 years research experience in oncology is required. Bench and practical experience in cell culture, cell cycle analysis, ELISA, biochemical techniques, enzymology, and/or in vivo models. PhD/MD is highly desirable.

Associate Scientist/ Sr. Associate Scientist–Oncology

Raritan, NJ (Req. 0501596)

The candidate selected for this position will be responsible for the planning, execution, and analysis of in vitro assays and in vivo animal models designed to demonstrate the efficacy of potential drug candidates in Oncology. Responsibilities will include the identification of molecular targets of disease, assay design and development, and the establishment of in vivo models. Experience in pharmacology and/or laboratory animal handling and experimentation is required. Work experience with tumor xenograft models and other oncology models is expected. BA/BS or higher in a scientific discipline. 3+ years experience with MS or 6+ years experience with a BS. A background in cancer research and experience with in vivo tumor models is required.

Post Doctoral Fellow–Bioinformatics

Raritan, NJ (Req. 0412290)

In this position you have the chance to serve as a joint member of both therapeutic teams and our bioinformatics team to apply your seasoned skills in life science research and other disciples to our drug discovery effort on biomarker initiative. You will be expected to have sound background in life science disciplines, especially metabolic disease, CNS, or Oncology. PhD in life science is required. Metabolic, CNS, or Oncological disease research and 0–3 years experience is preferred. Collaboratively work with therapeutic teams to work on biomarker discovery, identification, or validation through multidisciplinary approaches. Experience in bioinformatics and statistics is a plus.

Sr. Scientist/Principal Scientist–Biology

Exton, PA (Req. 0410418)

A cell biologist is needed within the Lead Generation Biology group. Lead Generation supports many therapeutic area project teams during initial target characterization (analysis of constructs), HTS, hit profiling, and development of HTS positives into Leads for therapeutic targets. The successful candidate will design, implement, analyze, interpret results of cell-based assays for multiple target classes, evaluate new technologies, and present experimental results to therapeutic teams as part of an interactive research team. A PhD in molecular or cell biology or a closely related field plus 5 years pharmaceutical experience designing and validating cell-based assays both for HTS and for profiling hits arising from other methods of HTS is required, as are good communication skills and demonstrated supervisory experience.

Drug Discovery Scientist/ Sr. Scientist–Biology

Exton, PA (Req. 0413004)

A mechanistic enzymologist is within the Lead Generation Biology Group. Lead Generation supports many therapeutic area project teams during initial target characterization (analysis of constructs), HTS, hit profiling, and development of HTS positives into Leads for therapeutic targets. The successful candidate will lead a group responsible for designing, troubleshooting, and conducting in vitro enzymological experiments, and will present experimental results to therapeutic teams as part of an interactive research team. A PhD in biochemistry or a closely related field plus 5–10 years pharmaceutical experience is required, as are demonstrated expertise understanding enzyme mechanisms, good communication skills, and demonstrated supervisory experience.

Post Doctoral Fellow–Biology

Exton, PA (Req. 0502188)

A postdoctoral fellowship is available within the Lead Generation Biology (Exton, PA). Research will focus on developing calorimetry and/or chromatography as methods for characterizing drug-mediated changes in enzyme activity, with implementation of these techniques in support of drug discovery. Collaboration, innovation, and creativity are key to success; publication of findings in peer-reviewed journals is encouraged. This position requires a PhD in Biophysics, Biochemistry, Enzymology, or related field, with a demonstrated track record of success in the study of protein function. Expertise in calorimetric techniques and mechanistic enzymology is highly desirable. Excellent written and verbal communication skills are essential.

Visit www.jnj.com/careers for more information or to forward your resume. Please reference company and requisition number with all specific applications.

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MRC Laboratories, The Gambia

MRC Medical Research Council

Virologist/Viral Immunologist

MRC Laboratories, The Gambia is the Medical Research Council's largest establishment conducting laboratory, field-based and clinical research in a developing country. The Unit aims to improve the health of people in developing countries, by contributing to the development, testing and safe adoption of interventions aimed at reducing the burden of morbidity and mortality from infectious diseases.

We seek an experienced research scientist with a background in virology or viral immunology and an interest in one of the virus infections that are currently being studied at the Unit. These include: HBV, HIV-1, HIV-2, HTLV-1, CMV, EBV and measles virus infection. We are interested in receiving applications from candidates with a PhD in virology or viral immunology and a minimum of three years' post-doctoral experience. You will be a team leader with proven experience of training and supervising staff at different levels, a strong publication list and experience in preparing research proposals and budgets. Experience in molecular virology including viral load assays and diagnostic PCR, and/or cellular immunology would be an advantage.

A competitive salary will be offered within pay band 3 (which starts at £34,245 per annum), commensurate with qualifications and experience. For displaced staff the package will also include generous overseas allowances, furnished accommodation, flights and other benefits. The post will be for three years, extendable by mutual agreement.

Application forms and further details are available electronically from samantha.smith@headoffice.mrc.ac.uk Alternatively hard copies are available by leaving a message on 020 7637 6005 or by faxing Samantha Smith on 020 7637 0361.

The closing date for applications is 29th April. Interviews will be held in London in late May or June.

For further information about MRC visit www.mrc.ac.uk

MRC is an Equal Opportunities Employer

New York University

ASSISTANT PROFESSOR

Department of Basic Science and Craniofacial Biology

COLLEGE OF DENTISTRY

The NYU College of Dentistry seeks applicants for a full-time Assistant Professor in the area of HIV/AIDS. This position is either research track or tenure track, depending upon qualifications and interests. The ideal candidate will have a Ph.D. and post-doctoral training and have initiated a strong research program in (1) HIV pathogenesis, (2) HIV diagnostics or (3) oral aspects of HIV/AIDS. We are particularly interested in individuals with a background in HIV virology, biochemistry and/or molecular biology. This individual will be expected to have, or to obtain, extramural funding, collaborate with other research investigators at NYU and, if on the tenure track, participate in the teaching of basic science to dental students.

Qualified applicants should submit a cover letter, CV, a research statement and names of three references to: **Dr. Daniel Malamud, c/o Louis Terracio Ph.D., Associate Dean for Research, New York University, College of Dentistry, 345 East 24th Street, Room 1036W, New York NY 10010-4086.** *NYU appreciates all responses, but can only respond to qualified candidates.*

NYU is an Equal Opportunity/Affirmative Action Employer.

Faculty Positions in Computational and Genome Biology

Assistant/Associate/Full Professor Positions

The Computational and Genome Biology Initiative at Oregon State University invites applications for up to three 9-month, tenure-track positions (one senior position). These are cornerstone positions within an initiative to expand research and teaching in computational and genome-centered biology. Competitive salary and startup packages will be awarded to successful candidates. Because of the interdisciplinary nature of these positions, academic homes will be identified by best fit through consultation between successful candidates and prospective departments and colleges. Qualifications for assistant professor positions include an earned Ph.D. or equivalent in a field of biological, computational, mathematical or related science. Relevant research and post-doctoral experience in computationally intensive biology, genome science or systems-oriented biology are required. Candidates engaged in interdisciplinary research, and enthusiastic about teaching and developing community-wide resources, are desired. Preference will be given to candidates with a demonstrable commitment to promoting and enhancing diversity. For associate or full professor positions, a record of high-impact contributions to science as an independent investigator is required.

Strategic Initiative in Computational and Genome Biology

As one of six strategic initiatives for OSU, the Computational and Genome Biology Initiative intends to strengthen and expand interdisciplinary research and education in genome-centered and information-based areas of biology. The new faculty may focus on any of a broad range of areas, such as evolutionary genomics, epigenomics, computational imaging and modeling, and systems biology. The new faculty will participate in instruction within the Molecular and Cellular Biology Graduate Program, and in the development of new core facilities or capabilities within the Center for Gene Research and Biotechnology The Initiative will further strengthen recent investments in computational, genomic, proteomic and imaging facilities in the CGRB and partner centers at OSU. For additional information, see **http://applications.cgrb.oregonstate.edu/**. All applications must be submitted using the secure online system.

Contact
James C. Carrington,
Initiative Coordinator
cgbi@cgrb.oregonstate.edu
For full consideration, applications must be received by May 23, 2005.

Oregon State UNIVERSITY

OSU is an AA/EOE, and has a policy of being responsive to dual-career needs

Max Planck Institute for Demographic Research

Directors: Prof. James W. Vaupel - Prof. Jan M. Hoem

The Max Planck Institute for Demographic Research
is seeking to expand further its activities in the field of Evolutionary Biodemography
and is recruiting to

PhD, Post-Doc, and Research Scientist level
vacancies involving the

Mathematical Modeling of Life History Evolution.

The successful candidates will complement an existing research team of 19 staff, including a number of recently recruited evolutionary ecologists, and will work alongside a total of some 80 employees from diverse backgrounds engaged in a range of issues in demography. The team aims to gain a fundamental understanding of how age-specific demographic processes are shaped by evolution. We have ongoing projects on age-specific schedules of mortality, reproduction and growth, on the evolution of senescence, on reproductive effort, parental investment and intergenerational transfers, on environmentally-cued life-history choices, and on the costs of reproduction and the delayed effects of stress. We seek to advance our knowledge of these issues using a combination of theoretical modeling, analysis of existing databases, and a variety of field and laboratory based studies. We wish to complement our empirical studies with theoretical insights through the appointment of scientists engaged in the mathematical modeling of fundamental evolutionary processes.

We are seeking able scientists from all levels with strong academic track records in quantitative disciplines. Those with backgrounds in mathematics or quantitative life history modeling are particularly encouraged to apply. Applications should be addressed to Executive Director, Prof. James W. Vaupel and should include a CV with a statement of academic interests and relevant experience, qualifications, a list of publications and the contact details of 3 referees. All material should be e-mailed to: **math-evol.positions@demogr.mpg.de.** See www.demogr.mpg.de for information.

The Max Planck Society wishes to increase the share of women in areas where they are underrepresented, and strongly encourages women to apply.

The Max Planck Society is committed to employing more handicapped individuals and especially encourages them to apply.

Math-Evol Positions, Attn. Prof. James W. Vaupel
Max Planck Institute for Demographic Research
Konrad-Zuse-Straße 1, D-18057 Rostock, Germany
E-mail: math-evol.positions@demogr.mpg.de

MAX PLANCK SOCIETY

DIRECTOR

WORLD CLIMATE RESEARCH PROGRAMME

The World Meteorological Organization (WMO) invites applications for the post of Director of the World Climate Research Programme (WCRP) Department. The incumbent is responsible on behalf of the sponsoring bodies (WMO, ICSU and IOC) for the international coordination, planning and organization of scientific research projects and related activities contributing to the goals of the WCRP programme. The incumbent represents the interests of the programme with relevant governmental and non-governmental international organizations and a wide range of national administrations and research agencies. He/she will need to establish effective working relationships with a wide international community of scientists in the fields of meteorology and atmospheric sciences, oceanography, polar sciences, hydrology and land surface processes, space research. In accordance with the terms of the WMO/ICSU/IOC Agreement on the WCRP, the incumbent will be responsible for the scientific and technical tasks discharged by the JPS to the Chairperson of the Joint Scientific Committee for the WCRP and for financial and administrative matters, to the Secretary-General of WMO.

Applicants will have Ph.D or equivalent qualification in the fields of meteorology, atmospheric sciences, oceanography, polar sciences, hydrology or space science. Over ten years experience including international research recognition in climate-related studies and global environmental change. Experience in planning and organizing large scientific projects and/or management of a scientific institute preferably with international components. Excellent knowledge of English and/or French required.

Deadline for applications 10 May 2005. Personal History Forms, as well as additional information concerning the position, salary and benefits, can be obtained from the

WORLD METEOROLOGICAL ORGANIZATION
C.P. 2300, 1211 Geneva Switzerland
www.wmo.int (vacancy notices)

Faculty Position in the Field of Epitaxial Growth
Institute for Nanoscale and Quantum Engineering, Science and Technology
The University of Virginia

The Institute for Nanoscale and Quantum Engineering, Science and Technology (NanoQuest) at the University of Virginia invites application for a tenure track faculty position in the field of epitaxial crystal growth. This hire can be at the Assistant, Associate or Full Professor levels. The NanoQuest Institute was founded in 2002 to advance the university's activities in nanoscale and quantum science and technology. Further information may be found at **www.nanoquest.virginia.edu**.

The field of epitaxial crystal growth plays a key role in the university's current and planned activities, including a NSF Materials Research Science and Engineering Center (**www.mrsec.virginia.edu**). The successful candidate will be expected to head an internationally recognized research program in this field, providing interdisciplinary leadership across the university. He or she will have a primary appointment in the appropriate academic department within the University, but will also have a joint appointment within the Institute, and will be expected to provide substantial leadership in development of the Institute's research and education initiatives.

Candidates should possess a doctoral degree in a relevant discipline, should have a demonstrated ability to lead an internationally recognized research program, and should demonstrate commitment to excellence in teaching at the graduate and undergraduate levels. In addition, we seek candidates with the ability to build bridges between different academic disciplines. Applications from female and minority candidates are particularly encouraged.

Outstanding facilities for nanoscale assembly, synthesis, processing and characterization exist at the University, including a state of the art electron microscopy facility, several focused ion beam systems, multiple deposition systems, electronic materials processing, lithography and device fabrication laboratories, a state of the art ultra-fast laser facility, and extensive surface analysis instrumentation. A major new building for materials research and nanotechnology will be completed by mid 2006.

Selection of candidates for interview will commence around **June 1, 2005**, but the search will remain open until a successful candidate is identified. Candidates should send a curriculum vitae, statement of research interests, summary of research accomplishments to date, a statement of teaching achievement and philosophy, and the names of five references familiar with their teaching and research achievements to: **Professor Robert Hull; Director, NanoQuest Institute; 116 Engineers Way; Charlottesville, VA 22904**.

The University of Virginia is an Equal Opportunity/Affirmative Action Employer. Applicants must be able to lawfully accept employment in the United States.

Department of Health and Human Services
National Institutes of Health
National Heart, Lung, and Blood Institute

MEDICAL OFFICER

The Department of Health and Human Services and the National Institutes of Health announce the recruitment of a Physician to serve in the Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute, to manage clinical programs in Blood Diseases. The incumbent will provide leadership in the administration of research grants, reporting on scientific progress, developing and managing clinical studies/trials, and identifying opportunities for future research in the areas of hemoglobinopathies and hemostasis/thrombosis.

Salary: The current salary range is $88,893 to $135,136. In addition a Recruitment Bonus and/or a Physician Comparability Allowance (PCA) of up to $30,000 per year may be considered. Excellent health, life, investment and personal leave benefits.

How to apply: This position is being advertised through **USAJOBS**, an automated, on-line recruitment system **www.usajobs.opm.gov** at vacancy announcement number **NHLBI-05-60968** which opens Monday March 28, 2005 and closes **Monday May 2, 2005**. Applicants (resume and questions) must be received by 11:59 p.m. Eastern Standard Time on the closing date of this announcement. U.S. citizenship is required.

DHHS and NIH are Equal Opportunity Employers. Applications from women, minorities, and persons with disabilities are strongly encouraged. The NHLBI/NIH is a smoke-free workplace.

POST-DOCTORAL POSITION
Mechanism of Calcium Influx

Position available immediately in the Secretory Physiology Section, GTTB, NIDCR, NIH, to study the mechanism and regulation of Ca^{2+} entry in exocrine gland cells. Current studies address the role of TRP channel proteins in cellular calcium homeostasis and fluid secretion (**http://wwwdir.nidcr.nih.gov/dirweb/gttb/sps.htm**). A multidisciplinary approach is being used including electrophysiology, biochemical and molecular techniques, as well as functional studies and *in vivo* gene transfer in various animal models.

Candidates must have a significant publication record, and documented experience in molecular and cell biology techniques. Prior experience with confocal imaging is highly desirable. Candidates must have a Ph.D. and less than three years of post-doctoral experience.

Send CV and three letters of reference to:
Indu S. Ambudkar, Ph.D.
Chief, Secretory Physiology Section
Building 10, Room 1N-113
NIH
Bethesda, MD 20892

Phone: 301-496-5298
Fax: 301-402-1228
Email: indu.ambudkar@nih.gov

UNIVERSITY OF CALGARY

Three Chairs in Energy and the Environment at the University of Calgary

As part of its expansion of research and education programs in this priority area, the University of Calgary invites applications/nominations for three Chairs in Energy and the Environment. U of C is a comprehensive medical/doctoral research university with just under 30,000 students and annual revenues of about $750 million. It is located in the Energy Capital of Canada and the nation's fastest growing major city. Calgary ranks second in Canada in terms of head offices and first in terms of average level of education and income (see www.calgaryeconomicdevelopment.com and www.finance.gov.ab.ca for more information about the city and province).

We seek applicants with notable records of achievement in three related areas of energy and environment who would contribute to the mission of the University and the Institute for Sustainable Energy, Environment & Economy (see www.iseee.ca).

Svare Chair in Energy Systems Analysis

The successful applicant will be a distinguished scholar with an international reputation commensurate with an appointment at the rank of Professor (with tenure) and with strong teaching and leadership skills. Applicants must have a doctoral degree in the natural sciences, engineering or social sciences (e.g., economics, geography, business, law) and will be given an appointment in the appropriate academic unit(s) at the University. Substantial experience in applied energy policy or technology assessment and a strong record of interdisciplinary collaborations and publications are essential.

Two Canada Research Chairs (Tier II)

Candidates must demonstrate research excellence, as well as strong teaching, communication and leadership skills. They must have a doctoral degree (awarded after 1994) and will be given tenure-track appointments in the appropriate academic unit(s) at the University. Candidates should have relevant publications in top journals associated with these fields.

CRC (II) in Economics of Energy and Climate Change

We seek an individual with background in the social sciences (e.g., economics, geography, business, law), natural sciences or engineering. Expertise in risk and decision analysis, energy economic modelling, computable general equilibrium modelling, integrated assessment models of climate change or stochastic-dynamic programming will be an asset.

CRC (II) in Energy Engineering

We seek an engineer or scientist with expertise in energy technologies, combined with substantial applied experience in developing and deploying such technologies and with demonstrated interest in energy systems analysis. A focus in one of the following technical areas will be an asset: CO_2 management; alternative energy sources (e.g., wind and solar power); alternative transportation fuels; electric power systems; emission control technologies; or energy efficiency.

To Apply or Nominate, see full application/nomination details at: http://www.iseee.ca/whatsnew/employment/employment1.shtml
Consideration of applications/nominations will begin **April 16, 2005**. Applications will be accepted until the positions are filled.

All qualified candidates are encouraged to apply; however, for the Svare Chair, Canadians and permanent residents will be given priority.

The University of Calgary respects, appreciates and encourages diversity.

To see all University of Calgary academic positions, please visit www.ucalgary.ca/hr/career and search by Job Families.

www.ucalgary.ca

Queens College

DIRECTOR, INSTITUTE TO NURTURE NEW YORK'S NATURE

The City University of New York (CUNY) invites applications for the position of Director of the Institute to Nurture New York's Nature at Queens College, part of CUNY's Urban Environment Initiative. Drawing on the combined resources of CUNY, the nation's largest urban university, the Institute is devoted to the protection of the natural landscape and habitability of New York City and environmentally sound management of its natural resources. For further information, see www.qc.cuny.edu/provost/Grad/NNYN.

The Director will be a prominent scientist with an earned doctorate, strong scholarly record, and distinguished reputation in environmental and/or ecological studies. He/she will be responsible for articulating and advancing the mission of the Institute, developing its public profile and outreach programs, stimulating new collaborations among faculty across the CUNY campuses, and identifying new sources of extramural funding. The Director will play a lead role in developing the University's Urban Environment Initiative, and will be tenured in an appropriate academic department at Queens College. It is expected that the Director will maintain his/her own research activities. Administrative experience in leading multi-investigator research is desirable. Applications should include a letter of interest that outlines qualifications for the position, a curriculum vitae, a statement of vision of the Institute, and names and contact information for five references. Applications, which will be accepted until the position is filled, should be sent to Dr. Marten denBoer, Associate Provost, Queens College, 65-30 Kissena Blvd., Flushing, NY 11367. Email submission preferred (mdenboer@qc.edu).

AA/EOE/IRCA/ADA

Academic Coordinator

Job Summary: The Mass Spectrometry Facility is a moderately sized program serving multiple constituencies nationally and internationally. It is a NIH/NCRR National Research Resource with five inter-related activities: technological innovation, collaboration with the biomedical research community, service, training and dissemination. It is supported with an annual budget of 3-4 million dollars and involved extensively not only within UCSF but also with scientists from around the world. The incumbent will administer, manage and coordinate the Mass Spectrometry Facility and act as liaison between the collaborator and user community of over 100 investigators. This is a dynamic, collaborative, academic research facility focusing on the application of mass spectrometry to biological questions.

Required Qualifications: The incumbent must have a Bachelor's degree and a demonstrated ability to understand and support the scientific research enterprise. They must possess demonstrated strong, collaborative, inter-personal skills with both staff and faculty on a local level as well as on a national and international level. They must have a minimum of five years management and supervisory experience with an academic research environment involving extramural funding of programs and facilities from federal and other external agencies. They must have demonstrated experience in conceptualizing and implementing the support necessary to carry out the mission of a medium scale multi-disciplinary academic research program. They must have demonstrated experience in project planning, preparation of research and facility grants, in meeting project goals and timelines, in report preparation. They must have demonstrated experience in fiscal management of pre- and post-award activities. Excellent organization and communication skills are required for this position.

Preferred Qualifications: Management experience within the UC system, especially UCSF; Demonstrated experience in organizing and coordinating large international symposia.

This position is available effective May 1, 2005. Salary level is dependent on successful candidate's qualifications. Please send curriculum vitae and contact information for three references to: **Kris Casler (confidential), Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446** or email **LCK@itsa.ucsf.edu**. Application deadline: **April 18, 2005**.

The University of California is an Equal Opportunity/Affirmative Action Employer. All qualified applicants are encouraged to apply, including minorities and women.

POSITIONS OPEN

DIRECTOR
Washington National Primate Research Center

The University of Washington seeks candidates for the position of Director of the Washington National Primate Research Center. The Director plays a leadership role in defining the objectives of the Center, in developing policies to achieve those objectives, and in assuring the Center's quality, academic vigor, and integrity.

The successful candidate will (1) be an established scientist with a doctoral degree in one of the health science disciplines, (2) be the recipient of peer-reviewed biomedical research support, (3) meet the requirements for appointment to an academic department in the University, and (4) have experience with research on non-human primates.

Applicants are requested to submit a letter of interest and recent curriculum vitae (including a list of peer-reviewed publications) and copies of three representative publications via e-mail to the address below. The search committee will begin to evaluate applications on June 1, 2005.

Dr. Sidney D. Nelson
Chair, Search Committee
Professor and Dean, School of Pharmacy
c/o Patti Rosendahl
Box 357330
University of Washington
Seattle, WA 98195
E-mail: directorsearch@bart.rprc.washington.edu
Website: http://www.wanprc.org/WaNprc/

The University of Washington is an Affirmative Action/Equal Opportunity Employer. The University is dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to teaching and working in a multicultural environment and strongly encourages applications from women, minorities, individuals with disabilities, and covered veterans. All University of Washington faculty engage in teaching, research, and service.

POSITIONS OPEN

The Department of Pharmacology, Toxicology, and Neuroscience at the Louisiana State University (LSU) Health Sciences Center in Shreveport invites applications for a 12-month, tenure-track faculty position as an **ASSISTANT/ASSOCIATE PROFESSOR.** The LSU Health Sciences Center in Shreveport has a reputation for excellence in research and in medical and graduate student education. We are seeking an individual with demonstrated research expertise in toxicology that complements ongoing research in the Department including: cellular and molecular mechanisms of toxicity, cancer chemoprevention, drug metabolism, and neurotoxicology. Candidates must have a doctoral degree in pharmacology or a related discipline and pertinent postdoctoral experience. Excellent core facilities exist within the LSU Health Sciences Center, the adjoining Biomedical Research Institute, and the Feist-Weiller Cancer Center. The position will be available in 2005. Applications will be accepted until May 31, 2005. Candidates should submit a letter of application, curriculum vitae, and a detailed description of research accomplishments and future plans, and provide contact information for three or more references to: **James Zavecz, Ph.D., Faculty Search Committee, Department of Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center, P.O. Box 33932, Shreveport, LA 71130.** *LSU is an Equal Opportunity/Affirmative Action Employer.*

HARVARD MEDICAL SCHOOL

POSTDOCTORAL POSITION available to study the molecular mechanisms of neurodegenerative diseases using multidisciplinary approaches (*Neuron* **42:**23–36, 2004; *Neuron* **45:**489–96, 2005). Recent Ph.D.s with strong background in molecular biology and biochemistry are encouraged to apply. Send curriculum vitae and three reference letters to: **Dr. Jie Shen (website: http://www.shenlab.net)** at **e-mail: jshen@rics.bwh.harvard.edu.**

POSITIONS OPEN

ASSOCIATE PROFESSOR/ PROFESSOR, TENURE TRACK
Program Leader in Cancer Pharmacogenetics
The Ohio State University

The Comprehensive Cancer Center and the Department of Pharmacology, College of Medicine and Public Health, Ohio State University (OSU), seek an experienced translational researcher to lead a new program aimed to link the broad expertise of the Cancer Genetics and Experimental Therapeutics programs of the Cancer Center.

This challenging and highly visible role requires demonstrated scholarship and experience in both disciplines, as well as a commitment to collaborations with investigators of diverse areas of expertise with the goal of expanding the already substantial base of peer-review funding of the Cancer Center. The successful applicant is expected to play a central role in the scientific design of early phase cancer trials in humans, as well as integrate genetics/genomics and experimental therapeutics with early and late phase disease-specific trials.

Candidates must have a Ph.D., M.D., Pharm.D., or equivalent degree. Both senior and mid-level scientists will be considered for this unique opportunity.

Please submit a letter, the names of three references, and curriculum vitae by August 31, 2005, to:

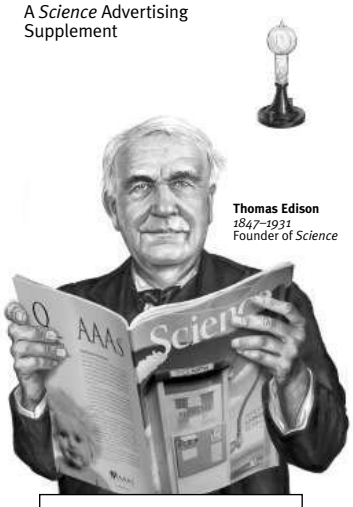
Miguel A. Villalona, M.D.
Associate Professor
Department of Medicine
The Ohio State University
B406 Starling Loving Hall
320 W. 10th Avenue
Columbus, OH 43210

For electronic submissions send to **e-mail: ring.32@osu.edu.**

OSU is an Equal Opportunity/Affirmative Action Employer.

Informatics Careers

A *Science* Advertising Supplement



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UNIVERSITY of VIRGINIA

Director of Blandy Experimental Farm

The University of Virginia's College of Arts and Sciences invites nominations and applications for the Director of Blandy Experimental Farm, a 700-acre research facility situated in the northern Shenandoah Valley, about 10 miles east of Winchester and 60 miles west of Washington, D.C. Blandy Experimental Farm is also the home of the State Arboretum of Virginia, which attracts approximately 75,000 visitors each year. Blandy's ambition is to achieve prominence as a regional, national and international center for environmental research and education. Blandy is unique among both public arboreta and university research units; it is a biological field station hosting research at the graduate and undergraduate levels, as well as being a classroom for citizens of all ages.

The Director will be located at Blandy Farm and serves as a 12-month, state-funded member of the research faculty in the Department of Environmental Sciences reporting to the Dean of the Sciences. The successful candidate must be qualified to hold a faculty appointment in the Department of Environmental Sciences and have a strong record of scholarship. The ideal candidate should demonstrate well-developed abilities in environmental education and fundraising, have strong experience creating and initiating educational and outreach programs for underserved populations, be able to work with diverse audiences, and build strong ties between research programs and outreach initiatives. The Director provides leadership for a staff including two research faculty who are permanent members of the Blandy staff, and five to ten faculty members who participate during the summer research season.

For further details about this position and links to information about the University of Virginia, the Department of Environmental Science, and Blandy Experimental Farm, please see **http://www.virginia.edu/blandy/director.htm**

Nominations are welcome and should include the name, position, address and telephone number of the nominee. Application materials should include a curriculum vitae with cover letter addressing how the candidates experiences match the position requirements, and contact information for three references. Submission of materials as a MS Word attachment is strongly encouraged. Application review will begin on April 18 and continue until the position is filled. Confidential inquiries, nominations and application materials should be directed to:

Professor Joseph C. Zieman, Chair of the Blandy Farm Director Search Committee
c/o Emily K. German
College of Arts and Sciences, University of Virginia
412 New Cabell Hall, P.O. Box 400771, Charlottesville, VA 22904
Phone: (434) 924-3437, Fax: (434) 924-1317
ekg9w@virginia.edu

The University of Virginia is an Equal Opportunity/Affirmative Action employer.

DEAN

ALBERT EINSTEIN COLLEGE OF MEDICINE of Yeshiva University

The Albert Einstein College of Medicine of Yeshiva University (AECOM) is seeking candidates for the position of Dean. This individual will also serve as Vice President for Medical Affairs of Yeshiva University and interact closely with the President and Vice President for Academic Affairs.

With its medical school, biomedical graduate school and post-doctoral training programs, AECOM is one of the nation's premier research-intensive medical colleges. In 2004 it was awarded in excess of $165 million in NIH funding and, with a new Center for Genetic and Translational Medicine under construction, the school is entering an exciting period of exponential growth. Renowned for the quality of its medical and graduate education, AECOM has an enviable record of training compassionate and world-class physicians and scientists. The school has an extensive network of clinical affiliates with the largest set of Graduate Medical Education programs in the country. Montefiore Medical Center is the College's primary affiliate. Other major academic affiliates include Jacobi Medical Center and the Bronx Lebanon Hospital Center in the Bronx, the North Shore-LIJ Health System in Long Island and Beth Israel Medical Center in Manhattan. Einstein's faculty is widely recognized for its collaborative efforts to further basic and translational science through its NIH-sponsored Centers for Cancer, Liver, AIDS, Neurodevelopment, Diabetes, and Sickle Cell Disease.

The College is now seeking a leader with a dynamic vision who will develop and implement a strategic plan that addresses its multiple missions and further strengthens its educational and research agenda. He or she must have an international reputation as an innovative and distinguished academic leader and investigator, be able to integrate basic, translational and clinical research programs, and have major expertise in the funding of biomedical science and education. The Dean must nurture and enhance the mutually beneficial relationships with clinical affiliates and facilitate cross-disciplinary coordination of research and educational enterprises. The candidate must have the qualities needed to work effectively to shape the future of the school in collaboration with a Board of Overseers committed to fundraising and philanthropy.

Interested applicants should write or email a letter of intent and C.V. to: **Dr. Matthew D. Scharff, Chairman, Search Committee for the Dean, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, 312 Belfer, Bronx, NY 10461, deansearch@aecom.yu.edu** We welcome applicants who will add diversity to our academic leadership and faculty. Equal Opportunity Employer

ALBERT EINSTEIN COLLEGE OF MEDICINE
Advancing science, building careers

POSITIONS OPEN

FACULTY POSITION
BIOINFORMATICS/ PHARMACEUTICAL SCIENCES
Center for Bioinformatics/
School of Pharmacy
University of Kansas

The School of Pharmacy and the Center for Bioinformatics within the College of Liberal Arts and Sciences at The University of Kansas invite applications for a jointly held tenure-track faculty position. This position is part of a new initiative created as part of a major expansion in the life sciences and will complement existing strengths, including structural biology, drug design, drug delivery, developmental/molecular genetics, protein analytical chemistry, and information technology. The appointment will be within one of the three science departments of the School of Pharmacy, Medicinal Chemistry, Pharmaceutical Chemistry, or Pharmacology and Toxicology, and the newly created Center for Bioinformatics. This Center for Bioinformatics/ School of Pharmacy interaction will foster international activities in bioinformatics and will combine outstanding research and the development of a first-rate Ph.D. program. Appointments are expected to be at the **ASSISTANT PROFESSOR** level. Duties: to establish and maintain an externally funded research program and to participate in teaching. Required qualifications: Ph.D. or equivalent degree, and demonstrated research activity and abilities in the area of protein interactions. We prefer candidates with research interests in protein biophysical, analytical, or physical organic chemistry and modeling of protein interactions. Examples include but are not limited to: protein docking; binding site prediction, energetics of binding and binding simulations; high-throughput modeling of protein structures; modeling of protein interaction networks; quantitative assessment of post-transnational degradation.

Mail or e-mail curriculum vitae, application letter, statement of research and teaching interests, and three reference letters to:

Dr. John F. Stobaugh, Ph.D.
Associate Dean
The University of Kansas
School of Pharmacy
1251 Wescoe Hall Drive
2056 Malott Hall
Lawrence, KS 66045-7582
E-mail: stobaugh@ku.edu

Direct inquiries to: **Dr. John F. Stobaugh, Ph.D.** at **e-mail: stobaugh@ku.edu.**

Review of applications will begin on May 22, 2005, and continue until the position is filled.

The University of Kansas is an Equal Opportunity/ Affirmative Action Employer.

ACADEMIC CARDIOLOGIST. The University of California, San Diego (UCSD) School of Medicine is actively recruiting for an Academic Cardiologist for a tenure-track/tenured position in the Division of Cardiology (**website: http://medicine.ucsd.edu/med**). Will consider both M.D. and M.D./Ph.D. candidates. The position will be primarily dedicated to performing basic research within the broad area of cardiovascular diseases and will also involve teaching of medical students, residents, and postdoctoral fellows. Effort as attending physician on the clinical services of the cardiology division will be expected. Candidates should have demonstrated productivity in basic research and acquisition of research support. Candidates must be board certified/eligible in cardiovascular medicine and be eligible for a California Medical License. Appointment level will be commensurate with experience and qualifications, and compensations based upon established UCSD salary scales. Interested individuals should send curriculum vitae and contact information for three references by April 28, 2005, to: **Kirk Knowlton, M.D., Chief, Division of Cardiovascular Medicine, University of California San Diego Medical Center, 200 West Arbor Drive, San Diego, CA 92103-8411,** or **e-mail: clgroves@ucsd.edu.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

NEUROSCIENCE INSTITUTE
Department of Neurosciences
College of Medicine
Medical University of South Carolina

The Medical University of South Carolina (MUSC) has implemented a strategic plan to expand areas of neuroscience research. Competitive startup support, laboratory space, and salary are offered. The Department of Neurosciences invites applications and nominations for the William Murray Endowed Professorship in Parkinson's disease, movement, and related disorders. The Murray Professorship is a tenured appointment at the **ASSOCIATE** or **FULL PROFESSOR** level. Applicants must have earned a Ph.D. or M.D. and be a recognized leader in neuroscience research. We are seeking applicants who have active research programs in the areas of Parkinson's disease and/or other movement disorders. The successful candidate is expected to be externally funded and establish a strong basic science and/or clinical research program. **Websites: http://www.neuroscience.musc.edu/** and **http://www.musc.edu/neuroscienceinstitute/.**

Applicants should apply online at **website: http://www.musc.edu/hrm/careers/faculty.htm.** Position requisition number is 041421. Applicants should also attach online a cover letter expressing their interest and qualifications along with curriculum vitae and three references addressed to: **Mark S. Kindy, Ph.D., Chair, Search Committee, Neuroscience Institute, Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, BSB 403, Charleston, SC 29425.**

MUSC is an Equal Employment Opportunity/Affirmative Action Employer.

TENURE-TRACK FACULTY POSITION
Pain, Stress, or Inflammation

The Department of Biomedical Sciences at the University of Maryland, Baltimore, is seeking candidates for a tenure-track faculty appointment. We encourage applications for positions at the **ASSISTANT PROFESSOR** level but will also consider applications from more **SENIOR INVESTIGATORS.** The applicant should have a Ph.D., postdoctoral experience, and an active research program. Preference will be given to a neuroscientist interested in the molecular, genetic, cellular electrophysiological, and/or imaging approaches to studying the neurobiological response to tissue injury. The individual filling this position will also be expected to participate in professional and graduate student educational programs. The successful applicant will have research space in a new state-of-the-art facility, to be occupied in July 2006. The University of Maryland has a world-renowned Program in Neuroscience, providing for interactions among the various basic and clinical neuroscience divisions, and linking neurosciences to other scientific disciplines within the University. Send curriculum vitae, statement of career objectives, and the names of at least three references to: **Dr. Joel Greenspan, Chair, Search Committee, Department of Biomedical Sciences, University of Maryland, Baltimore, 666 W. Baltimore Street, Baltimore, MD 21201. Website: http://bms.dental.umaryland.edu.** *We strongly encourage applications from qualified women and minority candidates.*

RNA POSTDOCTORAL POSITION, New York City, NIH-funded position is available at the City University of New York in the areas of RNA processing, transcription, and RNA interference/ microRNA. Applicants should have recent (zero to five years) Ph.D. in related field, including experience in cell culture, stable/transient transfection, protein purification, and molecular biology methods. Reply to: **Professor Kevin Ryan** at **e-mail: kr107@sci.ccny.cuny.edu.**

More information at **website: http://www.sci.ccny.cuny.edu/~kr107/index2/page5.html.**

POSITIONS OPEN

GENETICIST/ DYSMORPHOLOGIST

The Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University (MSU) announces a full-time, tenure-track position as an **ASSISTANT** or **ASSOCIATE PROFESSOR** in the Division of Human Genetics. The candidate must be board certified in pediatrics and board certified/eligible in clinical/ medical genetics. Qualified applicants will be expected to demonstrate the ability to conduct independent basic, clinical, or epidemiologic research. In addition, candidates will possess excellence in clinical diagnostic skills and teaching experience, commensurate with the appointment level sought. Letters of application and curriculum vitae should be sent to: **H. Dele Davies, M.D., M.Sc., Professor and Chair, Department of Pediatrics and Human Development, Michigan State University, B240 Life Sciences Building, East Lansing, MI 48824. E-mail: daviesde@msu.edu; fax: 517-432-4466.**

The due date for submission of letters of application and curriculum vitae is June 30, 2005, or until suitable candidates are found. *MSU is an Affirmative Action/Equal Opportunity Institution.*

UNIVERSITY OF PITTSBURGH AT JOHNSTOWN (UPJ): A four-year regional college, anticipates tenure-stream, full-time **ASSISTANT PROFESSOR** position to begin late August 2005. Responsibilities: 12 credits per term including introductory courses, research with undergraduates, and committee responsibilities. Requirements include: Ph.D. in biology, with concentration in developmental biology, and appropriate teaching and research experience. Review of applications will begin May 13, 2005, and continue until the position is filled. For full consideration, send (1) curriculum vitae, (2) statement of professional goals and interests including *statement of eligibility to work in the United States,* (3) statement of teaching philosophy, (4) evidence of quality teaching and research, (5) graduate transcripts (copies acceptable initially), and (6) three letters of recommendation to: **Dr. Stephen T. Kilpatrick, Biology Search Committee, Department of Biology, University of Pittsburgh at Johnstown, Johnstown, PA 15904.** Electronic applications will not be accepted. *UPJ is an Affirmative Action/Equal Opportunity Employer, and women and minorities are encouraged to apply.*

RESEARCH SCIENTIST

Stony Brook University seeks a Research Scientist. Required: Ph.D. in biological sciences with three years of postdoctoral experience in molecular biology. Incumbent will have the ability to independently design and perform research projects. Selected candidate will perform research in the development of vectors, development of transgenic mouse model, reporter, and electromobility shift assay, Western blotting, immuno precipitation, and transfection (transient and stable). Salary: $40,000 to $50,000. Please submit curriculum vitae and three letters of recommendation to: **Siamek Tabibzadeh, M.D., Department of Ob/Gyn, School of Medicine, Stony Brook University, Stony Brook, NY 11794-8091.** Visit **website: http://www.stonybrook.edu/cjo** for employment information. *Affirmative Action/Equal Opportunity Employer.*

SENIOR SCIENTIST POSITION

GeneThera, Inc., (Nasdaq OTCBB: GTHA) a biotechnology company located in Wheat Ridge, Colorado, seeks a job application for a Senior Scientist position. All applicants should have an M.D./Ph.D. or Ph.D. in molecular biology. Experience in the following areas is required: Real Time Fluorogenic-Polymerase Chain Reaction, Adenoviral Vector System, and RNA interference technology. Please send your curriculum vitae and names of three references to **e-mail: tmilici@genethera.net.**

Hundred Talent Faculty Positions
Institute of Microbiology, Chinese Academy of Sciences (IMCAS)

The research mission of the Institute of Microbiology, Chinese Academy of Sciences is to focus on biological sciences in microbiology resources, industrial and applied microbiology, pathogenic microbiology (including virology) and molecular immunology.

We are seeking outstanding scientists to join the institute as full professors and to develop a strong independent research program in molecular biology, cell biology, industrial microbiology, environmental microbiology, marine microbiology, pathogenic microbiology, virology and immunology. Candidates should have a Ph.D and assistant professor position or related postdoctoral training and the potential for outstanding achievement in the related field. It is expected that the successful candidate would lead the program in collaboration with members of the Institute. Tenure-track faculty appointments will be made in an appropriate academic program with start fund and generous salary. State -of- the- art space and equipment are available in new building near Beijing Asian Games Village. Electronic applications, including *curriculum vitae*, a brief statement of research interests and plans, and the names and contact details of two referees should be sent to: Ping Cheng, Institute of Microbiology, Chinese Academy of Sciences, P.O.Box 2714, Beijing 100080,China. Email: chengp@sun.im.ac.cn
Tel: +86-10-62554592, Fax: +86-10-62560912

Additional information on Institute of Microbiology, Chinese Academy of Sciences and its programs may be found at **www.im.ac.cn**.

NIAAA
National Institute on Alcohol Abuse and Alcoholism

Department of Health and Human Services
National Institutes of Health
National Institute on Alcohol Abuse and Alcoholism

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the National Institutes of Health and those of NIH's research Institutes.

The National Institute on Alcohol Abuse and Alcoholism (NIAAA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is seeking a Section Chief to direct the **Section on Model Synaptic Systems** within the Laboratory of Molecular Physiology, Division of Intramural Clinical and Biological Research. This is a **tenure-track** position equivalent to an Assistant Professorship in a University.

Applicants should hold a Ph.D. and/or M.D. degree and have a minimum of three years postdoctoral research experience. Significant resources are available to establish, staff, and operate an independent research program focused on investigating cellular and molecular mechanisms underlying synaptic transmission. The ideal applicant will have expertise in combining modern genetic techniques with cellular physiology in a genetically tractable vertebrate model organism such as zebrafish. The newly formed Section will complement two existing Sections (Transmitter Signaling, headed by **Dr. Stephen R. Ikeda**, and Cellular Biophotonics, headed by **Dr. Steven S. Vogel**) comprising the Laboratory of Molecular Physiology. Investigators with an interest in ion channels, signal transduction, or exo/endocytosis as applied to mechanisms of synaptic transmission are especially encouraged to apply.

Interested candidates should submit a C.V., list of publications, a brief research proposal that reflects the applicant's research interests and plan for establishing an independent program, and names of three individuals who could be contacted for reference to:

Ms. Roberta Greif
Administrative Laboratory Manager, LMP
NIH/NIAAA/DICBR
Room 3054
5635 Fishers Lane MSC 9304
Bethesda, MD 20892-9304

Review of applications will begin **May 1, 2005**, however, we will continue to accept applications until a suitable candidate is identified.

Women, minorities, and persons with disabilities are strongly encouraged to apply.
The DICBR/NIAAA is a smoke-free environment.
DHHS and NIH are Equal Opportunity Employers.

CONFERENCE

Teaching Science and Mathematics in the 21st Century: Challenges and Solutions

All agree that American undergraduate students must improve their mathematical and scientific competence. The challenge for science and math educators is the need for teaching reform so that student learning is the goal rather than the transmitting of information. This conference will introduce faculty and students to the challenges and problems facing science and math educators as well as provide practical suggestions on how student learning can be improved. Finally, the scholarship of teaching/learning at a research university will be discussed.

Guest Speakers:

Deborah Allen, University of Delaware
Brian Coppola, University of Michigan
Melvin George, University of Missouri
E.F. (Joe) Redish, University of Maryland
James Zull, Case Western Reserve University

Sponsored by the College of Sciences and Mathematics
www.auburn.edu/cosam/symposium

Auburn University, Alabama
Wednesday, May 4, 2005 from 8 am to 5 pm
Science Center Auditorium

Email: **lindsgl@auburn.edu** to register or for additional information. Registration is free, but lunch will only be provided to those who register in advance. Registrations must be received no later than **April 29, 2005**.

MRC Medical Research Council

MRC Laboratory of Molecular Biology, Cambridge

Programme Leader-Track Position in Neurobiology

Scientists interested in developing an independent programme of basic research are invited to apply for a programme leader-track position in the Division of Neurobiology. We are particularly looking to appoint an individual whose research focuses on synaptic integration and/or advanced neuronal imaging. Other areas of interest to the Division include the molecular mechanisms of synaptic transmission, circadian rhythms and neurodegenerative diseases.

The Laboratory of Molecular Biology provides an excellent environment for hands-on research. The research is funded by the Medical Research Council and there are no teaching responsibilities. There is an extensive central support including electronic and instrumentation workshops, imaging and transgenic mouse facilities. Interactions across the four Divisions within the Laboratory are encouraged. Additional information about the Neurobiology Division and about the Laboratory as a whole may be obtained at http://www2.mrc-lmb.cam.ac.uk

Further information can be obtained from Dr. Nigel Unwin (Tel: +44 1223 402492; e-mail: mas@mrc-lmb.cam.ac.uk) or Dr. Michel Goedert (e-mail: mg@mrc-lmb.cam.ac.uk)

Programme leader-track positions are equivalent to tenure-track and are for candidates at an early stage in their career. The appointment provides an opportunity over 6 years to demonstrate your suitability for a Programme Leader appointment. The starting salary is likely to be in the range of £34,245 - £40,252 per annum, depending on qualifications and experience. This is supported by a flexible pay and reward policy, and MRC final salary Pension Scheme. Some financial assistance with relocation may be available. We can offer 30 days annual leave entitlement and excellent on-site sports and social facilities.

To apply, please quote reference NB/305/6 and include a covering letter and CV with the names and addresses of two professional referees who can be contacted prior to interview.

E-mail your application to: recruit@mrc-centre.cam.ac.uk or post to Recruitment Office, Personnel Department, MRC Centre, Hills Road, Cambridge, CB2 2QH.

Closing date: 29 April 2005.

The Medical Research Council is an Equal Opportunities Employer.
'Leading Science for Better Health'

POSITIONS OPEN

COMPUTATIONAL BIOSCIENCES GROUP LEADER

Sandia National Laboratories (**website: http://www.sandia.gov**) has an immediate opening for a Group Leader in computational biosciences at our Albuquerque, New Mexico, facility. The Group Leader will join Sandia's leadership team as part of our larger bioscience and biotechnology effort, and will lead a department of scientists responsible for developing and applying unique computational biology tools. The position's responsibilities include identifying opportunities for applying Sandia's unique capabilities to life science challenges via highly collaborative partnerships. These capabilities include not only world-class computing resources, and long-standing research programs in mathematics, computer science, modeling, and simulation, but also in nanobiotechnology, advanced measurement and imaging science, and micro/nanosystems research and development. Research knowledge and leadership experience relevant to the mission of the group is required; this may include developing and applying computational tools for life science applications and biology and computational biology technical knowledge as evidenced by peer-reviewed publications. A proven record of research excellence, developing/managing funded research programs, and leading teams of researchers is essential, as are proven analytical, written, interpersonal, and presentation skills. Self-motivation and an affinity for working in a diverse team environment are necessary. Salary is commensurate with experience. Please submit resumes via e-mail (subject: Comp-Bio Group Leader) to: **Dr. Grant S. Heffelfinger** at **e-mail: gsheffe@sandia.gov.** *U.S. citizenship normally required. Equal Opportunity Employer. Minorities/Females/Persons with Disabilities/Veterans.*

POSTDOCTORAL POSITIONS–molecular/cell biology of bacterial toxins. Postdoctoral positions are available in the laboratory of **Dr. Randall Holmes** at the University of Colorado Health Sciences Center. We are currently investigating the structure, function, and trafficking of cholera toxin and E. coli heat-labile enterotoxins, characterizing the roles of the metalloregulatory proteins DtxR and IdeR in iron-dependent regulation of gene expression, and virulence in *C. diphtheriae* and *M. tuberculosis*, respectively, and developing structure-based methods for treatment and prevention of diseases caused by these important bacterial pathogens. Research facilities, grant funding, and training environment are excellent, and we have recently moved to new research laboratories. Recreational facilities of the beautiful Rocky Mountains are easily accessible. Salary is commensurate with training and experience. Submit curriculum vitae, bibliography, and names of three professional references to: **Dr. Randall Holmes, University of Colorado Health Sciences Center at Fitzsimons, Microbiology Department, Mail Stop 8333, P.O. Box 6511, Aurora, CO 80045.** *The University of Colorado Health Sciences Center is committed to Equal Employment Opportunity/Affirmative Action. Citizens and permanent residents of the United States may be considered for positions on an NIH-funded training grant, and individuals from underrepresented groups are encouraged to apply.*

ASSOCIATE RESEARCH SCIENTIST

Must be M.D. and/or Ph.D. with at least three years' experience in human clinical trials with a focus on hepatology, viral hepatitis, and HIV therapy. Must have transplant experience.

Contact: **Attention David,** by **fax: 212-305-6873.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

THE CHINESE UNIVERSITY OF HONG KONG

The Department of Biology invites applications for the posts of **ASSOCIATE PROFESSORS/ASSISTANT PROFESSORS** (reference 05/044(665)/2). Applicants should have (i) a Ph.D. degree in the biological sciences; (ii) postdoctoral research experience; and (iii) demonstrated record of research accomplishments. The appointees will (a) teach undergraduate and postgraduate courses (including advanced courses in their specialty); (b) either work on genomics or bioinformatics, or use a well-recognized model organism to address basic or applied biological issues; (c) capitalize on the well-established infrastructure in the Department for independent research in their fields of expertise, and collaborate with the existing research teams; (d) supervise postgraduate students; and (e) participate in administration. For information about the Department, please visit **website: http://www.cuhk.edu.hk/bio.** Appointments will initially be made on a fixed-term contract basis for up to three years, with prospect for renewal or a longer-term appointment thereafter subject to mutual agreement. Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefit package, including medical care, a contract-end gratuity for an appointment of two years or longer and housing benefits for eligible appointees. Further information about the University and the general terms of service for teaching appointees is available at **website: http://www.cuhk.edu.hk/personnel.** The terms mentioned herein are for reference only and are subject to revision by the University. Please send full resume, copies of academic credentials, a publication list, and/or abstracts of selected published papers, together with names, addresses, fax numbers, and e-mail addresses of three references to whom applicants' consent has been given for their providing references (unless otherwise specified) to: **The Personnel Office, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong** on or before 29 April 2005; **fax: 852-2603-6852.** The Personal Information Collection Statement will be provided upon request. Please quote the reference number and mark 'Application–Confidential' on cover.

ASSOCIATE CHIEF OF STAFF (ACOS) FOR RESEARCH AND DEVELOPMENT

The Veterans Affairs Medical Center (VAMC), Syracuse, New York, seeks an outstanding scientist with a vigorous research program to serve as ACOS for Research and Development. The Syracuse VAMC is an active teaching hospital (a 140-bed facility) affiliated with Upstate Medical University. The ACOS must have a strong record of funded research, experience fostering inter-institutional relationships, experience building thematic research programs, and administrative experience. The Syracuse VAMC has a long history of neuroscience research and will be the site of a $54 million spinal cord injury/disease center. Additionally, a $9 million grant has been received to support the Center for Integrated Healthcare to explore the integration of mental health and primary care services. The Syracuse VAMC expects to build on these strengths, but will consider strong applicants in any field of biomedicine. Applicants must possess a doctoral degree (M.D. and/or Ph.D.) and must qualify for a corresponding academic appointment at Upstate Medical University College of Medicine. *American citizenship or resident alien status is required.* Interested candidates should mail, **fax: 315-425-2447** or **e-mail: melissa.boak@med.va.gov** a cover letter and current curriculum vitae (including records of funding and mentoring) to: **Melissa Boak, Human Resources/05, VA Medical Center, 800 Irving Avenue, Syracuse, NY 13210.** Information on Upstate New York VISN 2 VA Healthcare Network can be found at **website: http://www.va.gov/visns/visn02.** Position is subject to random drug testing. *The VAMC is an Equal Opportunity Employer.*

POSITIONS OPEN

NEUROPATHOLOGY FACULTY POSITION

We are seeking an academic neuropathologist with a strong NIH-funded research program, or the potential to develop one, to join The Methodist Hospital Research Institute (TMHRI) and the Department of Pathology at The Methodist Hospital in Houston, Texas. The TMHRI is directed by **Michael W. Lieberman, M.D., Ph.D.,** who is also Chair of the Department of Pathology. Neuropathology at TMH is directed by **Suzanne Power, M.D.** and has an active diagnostic service (over 700 brain tumors/year) and a vigorous neurodegenerative disease research program. The Texas Medical Center is home to a vibrant and strongly interactive neuropathology community at The Methodist Hospital, Baylor College of Medicine, M.D. Anderson Cancer Center, and the University of Texas Health Science Center Houston. Substantial protected time, generous startup funds, and state-of-the-art resources will be available, but some required diagnostic neuropathology service work will be required.

Please send curriculum vitae and cover letter to:

Suzanne Powell, M.D.
Chief of Neuropathology
Department of Pathology, MS205
The Methodist Hospital
6565 Fannin Street
Houston, TX 77030
Telephone: 713-441-6486
E-mail: spowell@tmh.tmc.edu

FACULTY POSITION
Molecular Cardiology
Cleveland Clinic

The Cleveland Clinic's Molecular Cardiology Department seeks applicants with expertise in applying basic (molecular/cell biology) research techniques to heart failure. We invite inquiries for a Staff position (**ASSISTANT/ASSOCIATE/FULL PROFESSOR**) for Ph.D./M.D. candidates with a well-rounded basic-research track record; position involves opportunity to collaborate with an active clinical cardiology group specializing in heart failure and transplantation.

The Cleveland Clinic (**website: http://www.clevelandclinic.org**) offers competitive salaries and benefits. Its Lerner Research Institute (**website: http://www.lerner.ccf.org/**) comprises more than 1,100 scientists in eight interactive departments, with Core Services for scientific support. The Institute's Department of Molecular Cardiology (**website: http://www.lerner.ccf.org/moleccard/**) has 12 staff-level Principal Investigators, whose research efforts in cardiovascular disease range from studies at the molecular and cellular levels to transgenic animal models to bioinformatics.

Send a cover letter along with curriculum vitae and names of three to five references to: **Subha Sen, Ph.D., D.Sc., Chair, Search Committee/NB50, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. Telephone: 216-444-2056, fax: 216-444-3110, e-mail: sens@ccf.org.**

The Cleveland Clinic Foundation is an Affirmative Action/Equal Opportunity Employer.

LABORATORY TECHNICIAN

Stony Brook University seeks a Laboratory Technician. Required: Bachelor's degree in relevant discipline with one year of experience in molecular biology and tissue culture techniques. Incumbent will set up and carry out experiments involving tissue cultures, Northern blotting, Western blotting, and enzyme-linked immunosorbent assay under the supervision of the research scientist. Salary: $27,000 to $35,000. Submit curriculum vitae and three letters of recommendation to: **Siamek Tabibzadeh, M.D., Department of Ob/Gyn, School of Medicine, Stony Brook University, Stony Brook, NY 11794-8091.** Visit **website: http://www.stonybrook.edu/cjo** for employment information. *Affirmative Action/Equal Opportunity Employer.*

Department of Microbiology
Faculty Positions

The Department of Microbiology at the University of Pennsylvania's School of Medicine seeks candidates for several Assistant or Associate Professor positions in the tenure track. Rank will be commensurate with experience. The successful applicant will have experience in the field of Genomics with a focus on Infectious Disease. Applicants must have an M.D. or Ph.D degree and have demonstrated excellent qualifications in Research. In this context, "genomics" encompasess global, comprehensive, high-throughput, cost-effective approaches to studying biological systems. Appointments will be within the Microbiology Department, (http://www.med.upenn.edu/micro/faculty.html) and the Penn Genomics Institute (http://www.genomics.upenn.edu/default.jsp). Any genomics research program with some relationship to infectious disease is potentially suitable.

Please submit curriculum vitae and a brief statement of research interests by June 30, 2005 to:

Search Committee
c/o Anna Britt
Department of Microbiology
3610 Hamilton Walk
Room 402 Johnson Pavilion
Philadelphia, PA 19104-6079
abritt@mail.med.upenn.edu

The University of Pennsylvania is an affirmative action/equal opportunity employer and is strongly committed to diversity. Minorities/Females/Individuals with Disabilities/Veterans are encouraged to apply.

Faculty Positions in Microbiology and Immunology
Department of Microbiology
University of Minnesota

The Department of Microbiology at the University of Minnesota Medical School invites applications for two faculty positions to be filled at the tenure-track Assistant Professor or tenured Associate Professor level. We are searching for outstanding scientists in two areas: (1) bacterial virulence and immune responses against bacteria and (2) HIV virulence and immune responses to HIV. Scientific excellence is the main criterion for selection. Successful applicants at the Assistant Professor level will be expected to establish a productive, independent research program, and successful applicants at the Associate Professor level should already be productive, established scientists. Successful applicants will also need qualifications and desire to contribute to departmental efforts in teaching medical microbiology at the undergraduate, graduate, and professional levels. The Department and affiliated units at the University have research strengths in several areas of microbiology, including immunity and host defense, pathogenesis, biodefense and emerging infectious diseases, microbial physiology, genetics, molecular biology, genomics, environmental microbiology and biotechnology. For more information about the Department of Microbiology, Center for Immunology, and the graduate training program, please visit: **http://microbiology.med.umn.edu**, **http://www.immunology.umn.edu**, and **http://micab.umn.edu**.

Minimum qualifications: M.D., D.V.M., or Ph.D. in microbiology or related discipline and extensive postdoctoral or faculty experience. Review of applications will begin on **May 15, 2005**, and continue until suitable candidates are identified. To apply, please submit a curriculum vitae and concise summaries of research and teaching interests and activities, and arrange to have three letters of recommendation sent to: **Patrick M. Schlievert, Ph.D., Search Committee Chair, Department of Microbiology, University of Minnesota, MMC 196, 420 Delaware Street S.E., Minneapolis, MN 55455**. Top candidates will be invited for a seminar/interview as a component of the selection process.

The University of Minnesota is committed to the policy that all persons shall have equal access to its programs, facilities, and employment without regard to race, color, creed, religion, national origin, sex, age, marital status, disability, public assistance status, veteran status, or sexual orientation.

MEETINGS

An Osprey enjoying early fall at the CSHL beach

Yeast Cell Biology
August 16 - 21 abstracts due: May 25

Eukaryotic mRNA Processing
August 24 - 28 abstracts due: June 1

Mechanisms of Eukaryotic Transcription
August 31 - September 4 abstracts due: June 8

Eukaryotic DNA Replication
September 7 - 11 abstracts due: June 15

Microbial Pathogenesis & Host Response
September 14 - 18 abstracts due: June 22

Programmed Cell Death
September 21 - 25 abstracts due: June 29

RNAi
September 28 - October 2 abstracts due: July 6

Neurobiology of *Drosophila*
October 5 - 9 abstracts due: July 13

Genome Informatics
October 26 - 30 abstracts due: August 3

Target Definition & Vector Design for Molecular Medicine
November 10 - 13 abstracts due: September 2

Molecular Approaches to Vaccine Design
December 1 - 4 abstracts due: September 30

Rat Genomics & Models
December 8 - 11 abstracts due: October 7

Cold Spring Harbor Laboratory
Meetings & Courses Program
1 Bungtown Road, Cold Spring Harbor, NY 11724
Phone 516 367 8346 Fax 516 367 8845
Email meetings@cshl.edu http://meetings.cshl.edu

POSITIONS OPEN

POSTDOCTORAL POSITIONS–molecular microbiology and pathogenesis of bacterial and viral infections. NIH training grant-funded Postdoctoral positions are available at the University of Colorado Health Sciences Center to study molecular mechanisms of bacterial infections (with **Randall Holmes, Michael Vasil, Andres Vasquez-Torres,** or **Martin Voskuil**), molecular aspects of viral infections (with **Bruce Banfield, David Barton, Thomas Campbell, Robert Garcea, Donald Gilden, Kathryn Holmes, Jerome Schaack, Kenneth Tyler,** or **Linda Van Dyk**), molecular basis of innate immunity (with **Charles Dinarello, Sonia Flores,** or **Andres Vasquez-Torres**) or structural biology of microbial pathogenesis (with **Mair Churchill**). See **website: http://www.uchsc.edu/sm/microbio/** for information about many of our research programs.

Research facilities, grant funding, and training environment are excellent. *Applicants for these positions must be citizens or permanent residents of the United States.* Candidates with Ph.D. or equivalent research degrees must have experience in microbiology, bacteriology, virology, immunology, molecular biology, genetics, biochemistry, cell biology, structural biology, or a related field.

Candidates with M.D., D.V.M., or equivalent clinical degrees must have demonstrated competency for research related to our program. Compensation is determined by NIH policies. Submit curriculum vitae, bibliography, and names of three professional references to: **Training Program Director, University of Colorado Health Sciences Center at Fitzsimons, Microbiology Department, Mail Stop 8333, P.O. Box 6511, Aurora, CO 80045.** *The University of Colorado Health Sciences Center is committed to Equal Opportunity/Affirmative Action. Individuals from underrepresented groups are encouraged to apply.*

POSTDOCTORAL POSITIONS

The Department of Medicine at the Medical College of Wisconsin, Milwaukee, invites applications for Postdoctoral positions available immediately to study molecular and cellular mechanisms of kidney diseases. Research projects are funded to (1) understand the protective role of CYP450 metabolites in glomerular disease; (2) study the mechanisms of bovine kidney virus infection of kidney cells; (3) investigate the mechanism of anti-apoptotic effects of Cyclooxygenase-2; (4) explore the role of adaptor proteins and protein kinases in endothelin signaling. Requirements include a Ph.D. or M.D. degree and a sound background in biochemistry, cell biology, and molecular biology. Experience in virology is preferred for project two and with animal models for project three. Candidates interested in the first project should send their curriculum vitae plus names and telephone numbers of three references to: **Ellen McCarthy, M.D.** at **e-mail: emccarth@mcw.edu.** Candidates interested in other projects should send their applications to: **Andrey Sorokin, Ph.D.** to **e-mail: sorokin@mcw.edu.**

POSTDOCTORAL FELLOW
University of Texas Health Science Center at San Antonio

Applications are invited for a Postdoctoral position in the laboratory of **Dr. Peter Hornsby,** Department of Physiology/Barshop Institute for Longevity and Aging Studies. There are two possible areas of focus for the research project: (1) tissue-based biosensor development; (2) Wnt signaling in the adrenal cortex, using a cell transplantation model. Full details are available at **website: http://physiology.uthscsa.edu/Faculty/hornsby/hornsby.html.** Salary $40,000 per year. To apply please send curriculum vitae to **e-mail: hornsby@uthscsa.edu.** Important: When replying please state which area of research you are interested in and briefly explain why you feel your background is appropriate for the project.

The University of Texas Health Science Center at San Antonio is an Equal Employment Opportunity/Affirmative Action Employer. All postdoctoral appointments are designated as security sensitive positions.

POSITIONS OPEN

FACULTY POSITION, CANCER RESEARCH

The Department of Pharmaceutical Sciences of the Texas Tech University Health Sciences Center (TTUHSC) School of Pharmacy invites applications for a tenure-track faculty position at either the **ASSOCIATE PROFESSOR** or **FULL PROFESSOR** level to join our growing Cancer Research group. Applicants should have an established, extramurally funded research program in cancer biology, cancer therapeutics, and/or tumor immunology. Successful candidates will be expected to develop and maintain an active research program as well as teach Pharm.D. and graduate students. The Department has 22 full-time Ph.D., Pharm.D., and/or M.D. faculty with interests in cancer biology, brain/vascular, and pharmaceutical research (**website: http://www.ttuhsc.edu/sop/PharmSci/**). The Amarillo campus of TTUHSC includes the School of Pharmacy, the regional School of Medicine, and the Harrington Cancer Center. Cancer research is a focus of this campus. Competitive startup packages and space are available. Applicants must apply and submit documents online at **website: http://jobs.texastech.edu.** Please include curriculum vitae, a summary of research and teaching interests, and names and addresses of three references. For questions, contact: **Dr. Ming-Hai Wang, Search Committee Chair** at **e-mail: minghai.wang@ttuhsc.edu** or **telephone: 806-356-4015, extension 248.** *TTUHSC is an Equal Opportunity/Affirmative Action/Americans with Disabilities Institution. Minorities and Women are encouraged to apply.*

EDITORIAL ASSOCIATE
The National Academies
Washington, D.C.

The National Academies in Washington, D.C., is seeking an Editorial Associate to facilitate recruiting of original research submissions for the multidisciplinary research journal, the Proceedings of the National Academy of Sciences (PNAS) and related activities. A Bachelor's degree in the biological sciences, or related field or equivalent knowledge with three years of related professional experience is required. A Ph.D. in a biological science and publishing experience is highly preferred. Superior communication skills and attention to detail are essential as well as a demonstrated ability to prioritize, multi-task, and meet critical deadlines.

For additional information on this position and to apply online, please visit **website: http://www.nationalacademies.org.** Under Employment, view Current Opportunities by department—Proceedings of the National Academy of Sciences (PNAS)—Requisition Number 050051-6. *Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

ASSISTANT PROFESSOR, Foods and Health-The Department of Food Science and Nutrition, University of Minnesota; nine-month, tenure-track position. See **website: http://fscn.che.umn.edu/** for details. *The University of Minnesota is an Equal Opportunity Educator and Employer.*

POSITIONS OPEN

The University of Alaska Anchorage (UAA) Biological Sciences invites applications for two full-time **POSTDOCTORAL MOLECULAR BIOLOGY POSITIONS** to conduct research in viral hepatitis (one position) and environmental/molecular toxicology (one position). Candidate must have a Ph.D. in the biological or life sciences, e.g., biochemistry, microbiology, immunology, physiology, or other professional degrees (e.g., an M.D. degree with previous laboratory experience). Previous experience with mammalian cell culture, DNA sequencing and analysis; and real time polymerase chain reaction, amplification, and troubleshooting is preferred. The ability to work effectively with students, technicians, and fellow researchers in a laboratory setting; demonstrated evidence of scientific productivity and with at least one peer review or first author publication; and previous research that is applicable to this position is required.

Review of applications begins on April 21, 2005.

The complete vacancy announcement with qualifications, responsibilities, and specific application procedures is available on the UAA/Human Resource Services **website: http://www.uaa.alaska.edu/humanresources. Telephone: 907-786-4608.** *UAA is an Affirmative Action/Equal Opportunity Employer and Educational Institution. Applications for employment are subject to public disclosure under the Alaska Public Records Act.*

FACULTY POSITION

The Department of Molecular and Cellular Physiology invites applications for a tenure-track position at the level of **ASSISTANT/ASSOCIATE PROFESSOR.** Successful applicants will be expected to develop an independent, nationally funded research program. Research areas are open, but preference will be given to individuals with an interest and record of achievement in cardiovascular science, inflammation, and/or oxidative stress. Information about the departmental research focus is available at **website: http://www.shreveportphysiology.com.** A generous startup package and appropriate space will be offered. Applicants should have a doctoral degree and relevant postdoctoral experience. Applications will be reviewed as they are received until the position is filled. Send curriculum vitae and names of three references to: **D. Neil Granger, Ph.D., Boyd Professor and Head, Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932. Fax: 318-675-6005, e-mail: dgrang@lsushc.edu.** *Louisiana State University Health Sciences Center is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL FELLOWSHIP in molecular virology. Self-motivation and expertise in negative-strand RNA viruses and/or anti-viral innate immune responses are required to pursue cancer virotherapy research. Submit application with curriculum vitae and bibliography via e-mail to: **Savio L.C. Woo, Ph.D., Mount Sinai School of Medicine, New York City** at **e-mail: savio.woo@mssm.edu.** *Equal Opportunity Employer.*

TRAINING

MECHANISMS OF NEURAL DISEASE
Graduate and Fellowship Training

Opportunities for research training at either the **POSTDOCTORAL** or **DOCTORAL** level in an NIH-sponsored training program,"Molecular Mechanisms of Neurological Diseases," are available at the Weill-Cornell Graduate School of Medical Science. Research areas include neurodegenerative diseases, stroke, neuro-oncology, addictive disorders, neuropsychiatric diseases, retinal disorders, neural degeneration, developmental neurobiology, and diseases associated with development and aging.

Applicants may contact: **Dr. John Wagner** (director of the training grant) at **telephone: 212-746-6586** or via **e-mail: jawagne@med.cornell.edu.**

AWARDS

KUWAIT PRIZE 2005

Invitation for Nominations

The **Kuwait Foundation for the Advancement of Sciences (KFAS)** institutionalized the **KUWAIT Prize** to recognize distinguished accomplishments in the arts, humanities and sciences. The Prizes are awarded annually in the following categories:

A. Basic Sciences
B. Applied Sciences
C. Economics and Social Sciences
D. Arts and Literature
E. Arabic and Islamic Scientific Heritage

The Prizes for **2005** will be awarded in the following fields:

1. Basic Sciences *: Computer Science*
2. Applied Sciences *: Water Resources Development*
3. Economics and Social Sciences *: Economy of Information and Development in the Arab World*
4. Arts and Literature *: Role of Arabic Literature in European Literatures*
5. Arabic and Islamic Scientific Heritage *: Medical Science and its History*

Foreground and **Conditions** of the Prize:

1. Two prizes are awarded in each category:
   - A Prize to recognize the distinguished scientific research of a Kuwaiti citizen,
   And,
   - A Prize to recognize the distinguished scientific research of an Arab citizen.
2. The candidate should not have been awarded a Prize for the submitted work by any other institution.
3. Nominations for these Prizes are accepted from individuals, academic and scientific centers, learned societies, past recipients of the Prize, and peers of the nominees. No nominations are accepted from political entities.
4. The scientific research submitted must have been published during the last ten years.
5. Each Prize consists of a cash sum of K.D. 30,000/ - (approx. U.S.$100,000/-), a Gold medal, a KFAS Shield and a Certificate of Recognition.
6. Nominators must clearly indicate the distinguished work that qualifies their candidate for consideration.
7. The results of KFAS decision regarding selection of winners are final.
8. The documents submitted for nominations will not be returned regardless of the outcome of the decision.
9. Each winner is expected to deliver a lecture concerning the contribution for which he was awarded the Prize.

Inquiries concerning the KUWAIT PRIZE and nominations including complete curriculum vitae and updated lists of publications by the candidate with four copies of each of the published papers should be received before **31/10/2005** and addressed to:

The Director General
The Kuwait Foundation for the Advancement of Sciences - P.O. Box: 25263, Safat - 13113, Kuwait.
Tel.: (+965) 2429780 / Fax: 2403891 / E-Mail: prize@kfas.org.kw

POSITIONS OPEN

University of Massachusetts Medical School
UMASS

Tenure-Track Neuroscience Positions

The Brudnick Neuropsychiatric Research Institute (BNRI), established as part of the unprecedented research expansion at the University of Massachusetts Medical School, invites applications for two tenure-track positions at the level of Assistant/Associate Professor. The BNRI was established in 2000 as a division of the Department of Psychiatry and is committed to broad based research investigating basic neurobiological principles underlying psychiatric disorders. Faculty interests focus on a variety of neurobiological problems and psychiatric disorders, with a common theme in the neurobiology of addiction, and applicants whose interests focus on addiction are especially welcomed. The BNRI is integrated into the Interdepartmental Neuroscience Program, which provides opportunities for graduate training and interactions with a large group of multidisciplinary neuroscientists. The BNRI is housed in a state-of-the-art laboratory facility, which includes magnets for high resolution functional brain imaging. Successful candidates are expected to establish independent research programs and play an integral role in new program initiatives. The positions are highly competitive with regard to salary, start-up funds, and laboratory space.

Applicants should send a CV, statement of research interests, and names and addresses of three references to:

Dr. Steven Treistman, Director
Brudnick Neuropsychiatric Research Institute
University of Massachusetts Medical School
303 Belmont Street
Worcester, MA 01604
E-mail: bnri@umassmed.edu
www.umassmed.edu/bnri

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ANNOUNCEMENTS

Harold M. Weintraub Graduate Student Awards 2005

The Fred Hutchinson Cancer Research Center congratulates the following recipients of the 2005 Harold M. Weintraub Graduate Student Award in recognition of outstanding achievement during Graduate Studies in the Biological Sciences.

Fernando D. Camargo	Baylor College of Medicine
Alice E. Chen	Carnegie Institution of Washington
Irene A. Chen	Harvard University
Pamela F. Colosimo	Stanford University
Joshua James Gooley	Harvard University
Elissa A. Hallem	Yale University
Jeffrey S. Han	Johns Hopkins University School of Medicine
Robert J. Johnston Jr.	Columbia University
Zachary B. Lippman	Cold Spring Harbor Laboratory
Bret J. Pearson	University of Oregon
Vanessa Ruta	The Rockefeller University
David Sayah	Columbia University
Dianne S. Schwarz	University of Massachusetts Medical School
Amy Hin Yan Tong	University of Toronto
Ingrid Elizabeth Wertz	Washington University School of Medicine

The recipients will participate in a Symposium this spring honoring Hal Weintraub and his commitment to innovative science.

More information on this award can be found at:
http://www.fhcrc.org/basic/weintraub

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Our assay kits are just one of the many cell signaling solutions we offer to more than a million scientists, every day. From the widest selection of products to the best technical support, we're the research partner of choice around the world. When you need a better way to assay, we're the only partner you need.

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- Total and Phosphospecific Tau ELISAs
- Senescent Cell Staining Kit
- BACE1 Activity Assay Kit
- JNK/p38/MEK Activity Assay Kit

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